Norwegian Institute for Water Research

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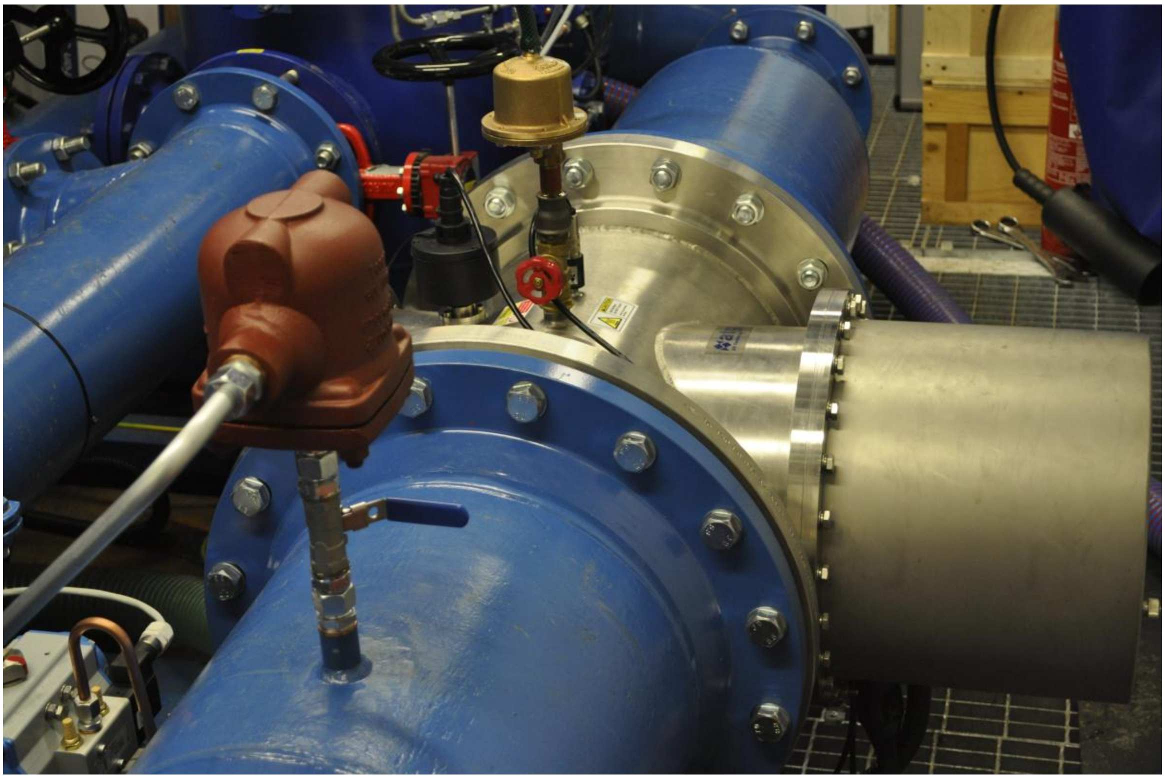
Land based testing of the Auramarine Crystal Ballast Water Management System.

Final Report.

Confidential



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REPORT

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Abstract

Land-based testing of the Auramarine Crystal Ballast water management system was performed from January to April 2010. The testing has been conducted according to the IMOs *Guidelines for approval of ballast water management systems (G8), Res. MEPC 174(58) Annex 4* and *Procedure for approval of ballast water management systems that make use of active substances (G9) Res. MEPC 169(57) Annex 1*.

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4. Auramarine	4. Auramarine

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Preface

The tests were conducted during winter and early spring in 2010 at NIVAs test facility located at Solbergstrand 20 km south of Oslo. NIVA conducted the testing as a contract assignment for Auramarine with the Norwegian administration represented by Det Norske Veritas (DNV, Norway) as verifier.

During planning and conducting of the tests, Juha Kiukas, Heikki Saaros, Jukka Suvanto and Kari Kautto were the lead representative of Auramarine. From NIVA, Stephanie Delacroix, Oddbjørn Pettersen, Per Ivar Johannesen, August Tobiessen and Helge Liltved were the main representatives. Several other Auramarine and NIVA personell have been involved in the project.

We will use the opportunity to thank Auramarine for choosing NIVA as the main partner in the process of testing and verification of the Crystal ballast water management system, and thank all involved personell for the professionalism demonstrated in completing this project.

Oslo, August 2010

Stephanie Delacroix

Contents

Abbreviations and acronyms

ACB-BWMS – Auramarine Crystal Ballast water management system
APHA – American Public Health Association
A. franciscana – Artemia franciscana
AOX – Adsorbable organic halogens
BWMS – Ballast Water Management System
CFDA-AM - 5-carboxyfluorescein diacetate acetoxymethyl ester
COD – chemical oxygen demand
CT2 – storage tank for control water
DBP – disinfection byproducts
DNV – Det Norske Veritas
DO – dissolved oxygen
DOC – dissolved organic carbon
DQIs – data quality indicators
EC_{50}, EC_{10} - the concentrations causing 50 and 10 % effect, respectively, on the test organism
Escherichia coli
EOX – Extractable organic halogens
EPA – Environmental Protection Agency (US)
FNU – Formazine Nephelometric Units
GC – gas chromatography
GF/F – glass fiber filter grade F
GLP – Good laboratory Practice
HAA – Haloacetic acids
IMO – International Maritime Organization
ISO – International Organisation for Standardization
LC_{50} – the concentration causing 50 % mortality of the test organism
LEL – Lower Explosive Limit
LLE – Liquid-Liquid Extraction
LOQ – limit of quantification
MSD – mass spectrometry detection
n – number of measurements; in calculating the standard deviation
NDIR – Nondispersive Infrared
NIVA – Norwegian Institute for Water Research
NS-EN ISO – Norwegian, European and International Standard
NTU – Nephelometric Turbidity Unit
OECD – Organisation for economic Co-operation and Development
PAR – photosynthetic active radiation
POC – particulate organic carbon
PSU – Practical Salinity Unit (= ‰)
QAPP – quality assurance project plan
QA/QC – quality assurance/quality control
QMP – quality management plan
S1-S5 – sampling points 1-5
SPE – solid phase exctraction
Std – standard deviation
TCBS - Thiosulphate citrate bile salt agar
T. suecica – Tetraselmis suecica
THM – trihalomethanes
TOC - Total organic carbon
TRO - Total residual oxidants
TSS – total suspended solids

TT1 – Tank for collection of deballasted treated water
TT2 – storage tank for treated water (5 day)/Tank for collection of deballasted control water
WST – tank with influent prepared test water
X_i – individual analytical result; in calculating the standard deviation
X – the arithmetic mean of individual analytical results; in calculating the standard deviation

Summary

Land-based testing of the Auramarine Crystal Ballast water management system (ACB-BWMS) was peformed from January to April 2010. The testing were conducted according to the IMOs *Guidelines for approval of ballast water management systems (G8), Res. MEPC 174(58) Annex 4* and *Procedure for approval of ballast water management systems that make use of active substances (G9) Res. MEPC 169(57) Annex 1*. The tests were carried out at NIVA’s test site located at Solbergstrand 20 km south of Oslo, using test water qualities according to the medium salinity (brackish water) and high salinity (seawater) defined by the guidelines. A total of 11 test cycles, five with brackish and six with seawater, were performed. Each test cycle lasted 5 days.

The test water was prepared in a 516 m^3 storage tank (WST). A combination of indigenous harvested organisms and cultured surrogate species (>50 µm group: *Artemia fransiscana*; 10-50 µm group: *Tetraselmis suecica*) were added to the test water to fulfil the water quality requirement regarding species diversity and organism density, respectively, within the >50 µm and 10-50 µm groups.

Each test cycle included ballasting and treatment of more than 200 m^3 of test water from WST at an average rate of 251 m^3/h for all test cycles. The treated ballast water was stored for five days in a storage tank (TT2), before it was treated once again during deballasting. The deballasted water was collected in a second storage tank (TT1) for sampling prior to final discharge. The treatment during ballasting included consecutive filtration and UV treatment, while the treatment during deballasting consisted only of UV treatment. For the first 10 test cycles the maximum (100 %) power input of the eight UV lamps were applied. For the additional test cycle with seawater (test cycle nr. 11) the UV power input was reduced by 50 %. Each test cycle was immediately followed by a control cycle where the same amount of test water (>200m^3) was pumped from WST at the same rate (ca. 250 m^3/h) using the same pump, but in by-pass of the ACB, to a parallel storage tank (CT2). After five days storage the control water was pumped to TT2 for sampling prior to discharge.

Fulfilment of the chemical and biological requirements of the initial test water

- The required levels for total suspended solids (TSS), dissolved organic carbon (DOC) and particulate organic carbon (POC) were fulfilled in all 11 test cycles.
- The requirements regarding organism density and diversity of the ≥50 µm group in the initial test water was met in all 11 test cycles.
- The requirements regarding organism density and diversity of the ≥10-50 µm group was met in all 11 tests.
- The requirement regarding concentration of heterotrophic bacteria in the test water was fulfilled in all 11 test cycles.

Fulfilment of the biological requirements of the control water at discharge

- The required more than 100 viable organisms ≥50 µm in minimum diameter per m^3 in the non-treated control water at the time of final discharge was fulfilled in all 11 test cycles.
- The required more than 100 viable organisms ≥10-50 µm in minimum diameter per ml in the non-treated control water at the time of final discharge was fulfilled in all 11 test cycles.

Fulfilment of the required biocidal effects at the time of final discharge of treated water

- The required less than 10 viable organisms ≥50 µm in minimum diameter per m^3 in the treated water at the time of discharge was fulfilled in all 11 test cycles.
- The required less than 10 viable organisms ≥10-50 µm in minimum diameter per ml in the treated water at the time of discharge was fulfilled in all 11 test cycles. This was documented by the serial dilution growth method and by the plate count method, both evaluating the organisms’ ability to reproduce. The CFDA vital staining technique, which assesses cell activity and membrane integrity, indicated more than 10 surviving oganisms in the test cycles

with medium salinity water and in the test cycle 11 with high salinity water. Almost all organisms observed as "alive" were *Tetraselmis* species which would have grown on the dilution growth method as well if they still had an intact reproduce capacity. However, the results indicated that the slow response of UV irradiation on cell activity gave false positive counts by using the CFDA-method; hence, the methods based on cell cultivation are regarded as more reliable and should be used for the evaluation of longterm viability.

- The required less than 250 cfu per 100 ml of *Eschericia coli*, less than 1 cfu per 100 ml of *Vibrio cholera* (serotypes O1 and O139) and less than 100 cfu of Intestinal *enterococci* per 100 ml at the time of discharge of treated water were fulfilled in all 11 test cycles.

Total residual oxidants (TRO)

TRO was measured as free and total chlorine (mg Cl_2/l) in prepared test water, in treated water and in control water on day 0 after ballasting, after 5 days storage and after deballasting on Day 5. Total residual oxidants (TRO) in the discharge water of the Auramarine BWMS at day 5 were below 0.02 mg Cl_2/l for all test cycles, and thereby below the recommended upper TRO limit of 0.1 – 0.2 mg Cl_2/l given by GESAMP (MEPC 58/2-8 GESAMP BWWG 7/9 Annex 4, 9.3.1).

Disinfection byproducts (DBP)

Influent water (from WST), treated water and non-treated control water in test cycles 1 and 2 (brackish water) and 6 and 7 (seawater) were sampled and analysed for adsorbable organic halogens (AOX), extractable organic halogens (EOX), bromate, trihalomethanes (THMs) and other halogenated organic and inorganic compounds as specified in the GESAMP DBP list (GESAMP-BWWG (MEPC 59/2/13 March 2009). The results indicated that no disinfection by-products were formed during treatment, neither for the brackish water tests nor for the seawater tests, as compared to the levels observed in the influent waters and the non-treated control waters.

Toxicity

A total of 18 toxicity tests with 6 different species and 5 different phyla have been performed on treated water prior to discharge. No evidence of toxic effect due to the treatment with the Auramarine Crystal Ballast water treatment system was detected in any of the performed toxicity tests. It is therefore unlikely that the treated and discharged ballast water will have any adverse effect in the recipient water upon deballasting.

1. Background

The goal for Auramarine is to get their Auramarine Crystal Ballast water management system certified according to the requirements in the IMO Convention on ballast water management (IMO, 2004) and the underlying guidelines; *Guidelines for approval of ballast water management systems (G8), MEPC 58/23/, Annex 4, Res. MEPC 174 (58), 200*, hereafter referred to as G8.

Land-based testing for type approval, as reported here, was conducted between January 20 and April 12 in 2010. The tests were conducted in accordance with G8.

2. Materials and test protocols

2.1 QA/QC procedures

Quality assurance and quality control have been performed during the testing according to Chapter 5 in the project specific QAPP and according to G8. All activities and data collected during testing of the Auramarine BWMS have been logged as summarized in **Table 1** using the data log sheets given in **Appendix A-K and P**. At the end of each test day, filled in and signed sheets were collected, saved in an electronic format and stored in NIVA's archive.

Table 1 List of log sheets used to document activities during testing.

Appendix	Description
A	Total project management
B	Chemical water quality preparation/Homogeneity
C	Biological water quality preparation
D	Operational data
E	Chemical analysis report
F	WST check list before each test
G	Logging of *in situ* measurements
H	Evaluation form for organisms ≥50 µm
I	Evaluation form for organisms ≥10-50 µm
J	Evaluation form for heterotrophic bacteria, coliforms, *E. coli*, Enterococcus group, intestinal *Enterococci*, *Vibrio cholerae* and *Vibrio cholerae* (serotypes O1 and O139).
K	Toxicity tests
P	Disinfection by-product analysis report

2.2 Test site

The tests were conducted at NIVA's test centre located at Solbergstrand 20 km south of Oslo. Seawater was supplied from various depths down to 60 m in the Oslofjord, while fresh water was supplied from ground water bore holes and from a local creek.

The test facility includes four glass-fibre reinforced polyester tanks, supplied with inlet and outlet arrangements and equipment for proper cleaning. See **Figure 1**. During storage of treated and control water, the tanks (TT1, TT2 and CT2) are covered to prevent exposure to light. The inner walls of the tanks are painted with ship coating (Balloxy HB light, Jotun, Norway). Propeller devices with gentle rotation are mounted at the bottom and at a shallow depth in WST and at the bottom in TT1, TT2 and CT2.

2.3 The evaluated ballast water treatment system

The Auramarine Crystal Ballast water management system (ACB-BWMS) was supplied by Auramarine. It combines pre-treatment by filtration and ultraviolet (UV) irradiation during ballasting, but by-passes the filtration unit during deballasting, hence at deballasting the water is only UV irradiated. For the first 10 test cycles the maximum (100 %) power input of the eight UV lamps were applied. For the additional test cycle with seawater (test cycle nr. 11) the UV power input was reduced to 50%.

2.3.1 General description of the Auramarine BWMS as described by the vendor

The following description is solely the product of the vendor and has not been subject to verification by NIVA:

The Auramarine Crystal Ballast treatment system is a two step process with an 40µm Boll&Kirch automatic filter to remove sediment and larger organisms followed by an 8-Medium Pressure UV lamps unit to disinfect and inactivate smaller plankton, bacteria and pathogens. Ballast water is treated during ballast water intake through the complete process and re-treated during deballasting by the UV reactor only (filter by-passed). Re-treatment during discharge is necessary to eliminate possible re-growth of bacteria in ballast tanks during storage and/or incomplete intake disinfection. The Auramarine Crystal Ballast system will be operated with a maximum feed water treatment capacity of 250m^3/h. Both process steps are carefully selected to provide a low pressure drop over the treatment system and a small and compact installation area.

2.4 Test waters

All test water (treatment run and control run) needed for each test cycle was prepared together in a 516 m^3 tank (WST).

2.4.1 Fulfilment of the chemical water quality test criteria

The chemical water quality criteria are shown in **Table 2**.

Table 2 Required chemical water quality of test waters as stated in regulation G8 by IMO. Salinities should be separated by at least 10 PSU

	Salinity	DOC	POC	TSS
Test water 1	>32 PSU	>1 mg/l	>1 mg/l	>1 mg/l
Test water 2	3-22 PSU	>5 mg/l	>5 mg/l	>50 mg/l

For the high salinity test water (>32 PSU) sea water from 60 meters depth was used. For the low salinity brackish test waters (approx. 22 PSU was envisaged) surface water from 1 m depth was used. Whenever necessary, fresh water from the nearby creek or local bore water was added to adjust the salinity.

In order to adjust the concentrations of dissolved organic carbon (DOC), particulate organic carbon (POC) and total suspended solids (TSS) to fulfil the requirements stated in **Table 1**, soluble lignin, starch and kaolin, respectively, were added. A full scale pre-test was conducted before commensing the main test cycles to find the appropriate amounts of these compounds to add to WST.

2.4.2 Fulfilment of the biological water quality test criteria

The biological water quality criteria are shown in **Table 3**.

A combination of indigenous harvested organisms and cultured surrogate species (>50 µm: *Artemia franciscana*; 10-50 µm: *Tetraselmis suecica*) were added to fulfil the biological water quality criteria.

Table 3 Required biological water quality in influent test water, treated water and in control water at the time of discharge as stated in regulation G8 by IMO

Organism group	Influent water	Treated water at discharge (Reg. D-2)	Control at discharge
≥50 µm min. dimension	Pref. 10^6 m^{-3}, ≥10^5 m^{-3} Min. 5 species from 3 diff. phyla/divisions	<10 viable organisms per m^3	> 100 viable organisms per m^3
≥10-50 µm min. dimension	Pref.10^4 ml^{-1}, ≥10^3 ml^{-1} Min. 5 species from 3 diff. phyla/divisions	<10 viable organisms per ml	> 100 viable organisms per ml
Heterotrophic bacteria	≥10^4 cfu ml^{-1}	-	-
Vibrio cholerae	-	<1 cfu/100 ml	-
Escherichia coli	-	<250 cfu/100 ml	-
Intestinal *Enterococci*	-	<100 cfu/100 ml	-

Tetraselmis suecica is a quite robust algae with an outer shell composed of cellulose-like material. It has a good survival when exposed to shear forces in pumps, and a good survival in the dark. In addition, it is tolerant with respect to survival in brackish seawater. Measurements by NIVA shows that *T. suecica* has an average minimum diameter of 9.3 µm (n=25) when growing exponentially in our cultures. *T. suecica* is therefore regarded as a robust representative of the type of organisms to be expected in the 10-50 µm size fractions of marine organisms.

Cultivation of Artemia franciscana and Tetraselmis suecica:

A. franciscana: Resting cysts of *Artemia franciscana* are available commercially. Hatching of cysts were achieved by adding approximately 1 g of cysts to 1 litre of 25 PSU seawater. The culture was incubated with a bright light source at a temperature of 22-26 °C with good aeration in the medium. Full hatching was achieved within 48 hours. It was possible to hatch approximately 100 000 nauplii per litre. The *Artemia* nauplii were hatched with a supply of food (egg yolk) and would therefore be alive for up to a week without any external food supply. Survival length is twice that if the nauplii had been stored at 8-10 °C. However, nauplii should be used within 2-3 days in order to achieve high survival and viability.

T. suecica: The algae are grown autotrophically in seawater growth media with added nutrients. The seawater is disinfected by filter (200µm, 50µm and 0.2µm) and UV radiation before use. The algae culture method used is the “growing culture volume” technique for isolated algae strains which are always available from NIVA’s algae culture collection. Large volume cultures need gentle aeration in order to maintain satisfying oxygen levels, bright light and controlled temperature. Densities of >10^9 per 1000 ml is reached after 7 days pre-culture and 7 days tank culture.

The necessary cultivation volume were calculated based on a final density (10^5 nauplii per litre for *A. franciscana* and >10^6 per ml for *T. suecica and*, final volume of the test water 500 m^3) and the desired concentration of the organisms in the test water (10^5 per m^3 for *A. franciscana* and 10^3 per ml for *T. suecica*.).

Harvesting of indigenous organisms

A Unik Filter type 450 (Unik Filtersystems, Os, Norway) equipped with a 20 µm mesh size screen was used to harvest indigenous algae and planktonic animal species from the fjord. This was done to assure the required presence of at least 5 species from 3 different phyla/divisions of both test groups of organisms in the test water (see **Table 3**). The harvesting process has been shown to be relatively gentle to the organisms; surface water (1 m depth) is smoothly pumped (ca. 3 m^3/h) by a jet-pump to the inlet side of the screen, and algae and animals are washed from the screen to a collecting tray (ca. 100 l/h, i.e. ca. 30 times enrichment) and transported to a storage tank with a volume of 10 m^3. The transport to the storage tank, and from the storage tank to the test tanks (WST), is designed to be as gentle as possible with a minimum of pumping. To document the fulfilment of the requirement for diversity of organisms in the test water, the variability (phyla, species) and general conditions of indigeneous harvested organisms were evaluated by microscopy. One sample was collected from the outlet pipe from the storage tank for harvested organisms during transfer to the WST tank, and triplicate samples directly from the WST tank after homogenisation.

Bacteria

The concentration of heterotrophic bacteria in the brackish test water is normally exceeding the required concentration of $\geq 10^4$ CFU ml^{-1}, while the heterotrophic bacteria criteria for the high salinity test water is expected to be fulfilled by the heterotrophic communities accompanying the cultured *Tetraselmis suecica* and *Artemia franciscana*.

2.5 Test cycle procedure

2.5.1 Description of a test cycle

The principle outline of one test cycle, which includes a treatment cycle and a control cycle, is shown in **Figure 1**.

The treatment cycle (blue lines): Each treatment cycle included ballasting and treatment by the Auramarine Crystal Ballast BWMS of more than 200 m^3 of test water from WST at an average rate of 251 m^3/h for all test cycles. The treated ballast water was stored for five days in a storage tank (TT2), before it was treated once again during deballasting. The deballasted water was collected in a second storage tank (TT1) for sampling prior to final discharge. The treatment during ballasting included consecutive filtration and UV treatment, while the treatment during deballasting consisted only of UV treatment. For the first 10 test cycles the maximum (100 %) power input of the eight UV lamps were applied. For the additional test cycle with seawater (test cycle nr. 11) the UV power input was reduced to 50%.

The control cycle (red lines): Each treatment cycle was immediately followed by a control cycle where the same amount of test water (>200m^3) was pumped from WST at the same rate (approx. 250 m^3/h) using the same pump, but in by-pass of the Auramarine Crystal Ballast BWMS, to a parallel storage tank (CT2). After five days storage the control water was pumped to TT2 for sampling prior to discharge.

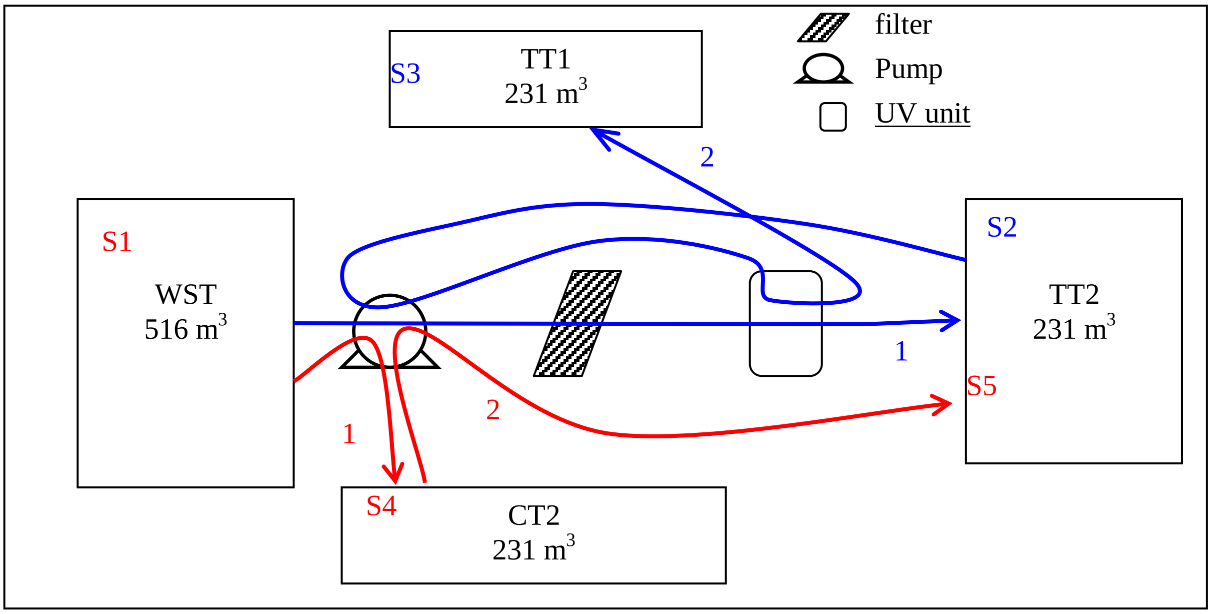

Figure 1 Transfer of test water during one test cycle with the Auramarine BWMS including a treatment line (blue) and a control line (red). Blue line 1 indicates the day 0 (ballasting) operation of treated water, whilst blue line 2 indicates the day 5 (deballasting) operation of treated water. Red line 1 indicates the day 0 (ballasting) operation of control water. Red line 2 indicates the day 5 (deballasting) operation of control water. Sampling locations and numbers are indicated by S1-S5 (red).

2.5.2 Assuring homogeneous distribution of particulate matter and stable load during treatment

The propeller devices in WST were used to ensure a homogenised distribution of suspended matter and, hence, assuring that the particulate load on the Auramarine Crystal Ballast BWMS was stable during operation. Therefore, measurements of turbidity at different points (periphery and centre, bottom, middle and upper part) in the water column of the tank of concern were conducted to verify homogeneity prior to commencing each test and prior to sampling. The TSS level was verified on site before commensing the test by sampling from the upper part of the periphery of the water column and using a rapid analytical method for quantification. Additionally samples were collected from the same location in the tank for accredited analysis for DOC, POC and TSS.

Similarly, before deballasting from TT2 (for the treatment cycle) and from CT2 (for the control cycle) on day 5 the propeller devices in these tanks were started a few hours before transfer to ensure a homogenized distribution of the suspended matter. Also here the homogeneity of the distribution was documentet by turbidity measurements at different points in the water column and sampling for DOC, POC and TSS analysis.

2.5.3 Control of system performance

A performance control of the total ballast water management system was conducted for every test cycle by the site responsible person from NIVA. Operational parameters for documenting the performance of the treatment technology were monitored and recorded; start and stop time for ballasting and deballasting, water levels in the tanks after each filling, water flows during operation, UV intensity inside the UV chamber and consumed energy by the Crystal Ballast BWMS during treatment (both ballasting and deballasting).

The water flow was measured by the internal flow meter in the ACB-BWMS and recorded every five minute by NIVA personell. Flow rates were also back calculated from the time it took to fill each tank with a known volume of water.

The UV intensity was measured by an internal UV meter in the UV chamber and read from the control panel of the ACB-BWMS and recorded every 5 min during operation. The quartz sleeves of the UV lamps were cleaned regularly by an automatic scraping system, but the UV sensor had to be cleaned manually. This was done twice; before deballasting on cycle 5 and before ballasting on cycle 10.

The energy consumed during ballasting and deballasting was monitored by NIVA by recording the energy input shown on NIVAs fuse cabinet inside Solbergstrand research station. The energy included all energy consumption connected with using the ACB-BWMS, excluding water pumping.

2.5.4 Measures to avoid cross-contamination during water transfer and sampling

To avoid cross-contamination between consecutive test waters upon transfer between tanks, all pipelines and tanks were flushed for 2-3 min with sea water from 60 meters depth or ground water with documented quality between each test cycle, followed by rinsing with high temperature water (80-90°C).

The same holding tank (TT2) was used for both treated water and control water in the same test cycle. To avoid contamination, treated water was always introduced to TT2 before control water. No cross-contamination was therefore possible to occur because the density of organisms was much lower in treated water than in control water. To avoid cross-contamination during sampling, the buckets, siphon overflow and plankton net were rinsed in sea water from 60 meters between each sampling.

2.6 Sampling

2.6.1 Assuring the representativeness of samples

To assure that representative samples were withdrawn from the tanks (WST, TT2 and CT2 at day 0, and from TT1 and TT2 at day 5) the turbidity in different sections of each tank (upper part, middle part and bottom part, in both periphery and center) was measured during homogenization and prior to sampling. The turbidity was measured by a handheld submersible probe (YSI – 600 OMS). When all turbidity measurements were within a 10 % deviation from the average turbidity of all measurements in the tank, sampling was commenced.

2.6.2 Sampling protocols

The following procedures were used to collect samples from the different tanks and sampling times. All samples were collected in triplicates.

1) *Sampling of bacteria in WST (S1), TT2(S2), TT1 (S3), CT2 (S4) and TT2 (S5)*: Bacterial samples were collected as 3x1000 ml (3x2x500 ml) ml grab samples by slowly submerging 500 ml sterile bottles and filling them with water from ca. 20 cm below the water line, assuring some leftover headspace in the bottle. The bottles had pre-added thiosulfate supplement (150 mg per litre) to neutralize any possible residual oxidants. The bottles were closed immediately after sampling and cooled at 4°C.
2) *Sampling of organisms ≥ 50 µm in WST (S1), CT2 (S4) and TT2 (S5).* A plastic bucket was used to collect 3x 20-100 litre sample. The sampled water was slowly sieved through a plankton net (50 µm diagonal dimensions) attached to a plastic cup, collecting the organisms in the cup. The sample from WST was also used for determination of required diversity.
3) *Sampling of organisms ≥ 50 µm in TT2(S2) and TT1 (S3):* A siphon spillway (gravity sampling hose) was used to collect 3x 1 m^3 test water from TT1 directly after transfer, and from TT2 after 5

days storage and deballasting. The water was sieved directly through a plankton net (50 µm diagonal dimensions) attached to a plastic cup, collecting the organisms in the cup. The sieved water was collected in a holding tank with known volume to ensure an accurate sampling volume.

4) *Sampling of organisms 10-50 µm in WST (S1), TT2 (S2), TT1 (S3), CT2 (S4) and TT2 (S5)*: Organisms with a minimum diameter between 10 µm and 50 µm were sampled as 3x 1000 ml for control water with clean glass bottle and 3x 10 litres for treated water with a clean plastic bucket. The sample from WST was also used for determination of required diversity.
5) *Sampling of water for pH, salinity, organic carbon measurements in WST (S1), TT2 (S2), TT1 (S3), CT2 (S4) and TT2 (S5)*: Water was collected as 3x 1000 ml grab samples by slowly submerging 1000-ml clean plastic bottles and filling them with water from ca. 20 cm below the water line, assuring some leftover headspace in the bottle. The bottle was closed immediately after sampling and cooled at 4°C.
6) *Sampling of water for TRO measurements:* Water was collected as a 1000 ml grab sample by slowly submerging a 1000-ml clean glass bottle and filling it with water from ca. 20 cm below the water line, assuring some leftover headspace in the bottle. The bottle had been pretreated with bleach to remove any chlorine demanding substances. TRO was measured immediately after sampling.
7) *Sampling of treated water for toxicity tests with algae and invertebrates in TT1 (S3):* Water was collected as 1000 ml grab samples by slowly submerging a 2 litre clean glass bottle and filling it with water from ca. 20 cm below the water line, assuring some leftover headspace in the bottle. The bottle was closed immediately after sampling.
8) *Sampling of treated water for toxicity tests with fish in TT1 (S3):* A gravity sampling hose connected to the siphon spillway was used to transfer treated water directly to several 300 L stainless steel storage tanks in a climate room where the fish toxicity tests were undertaken. This was done after finishing the general sampling routine on day 5.
9) *Sampling of test water for DBP measurements:* Water was collected as 6x 1000 ml grab samples by slowly submerging 1000-ml ALS glass bottles and filling them completely with water from ca. 20 cm below the water line. The bottles had pre-added thiosulfate supplement (150 mg per litre) to neutralize any possible residual oxidants. The bottle were closed immediately after sampling and cooled to 4°C.
10) *Sampling of sludge in the backflush water from the filter:* Sludge in the backflush water were collected as 2 liter grab samples directly from the sampling point on the pipe line between the filter and the storage tank for backflush water.
11) *Sampling of organism ≥50 µm and ≥10-50 µm for diversity analysis:* In addition to the diversity-analysis of samples from WST mentioned in point 2) and 4), the organism diversity was also analysed in water collected from the storage tank for harvested indigeneous organisms (see 2.4.2) and slowly sieved through a plankton net (50 µm diagonal dimensions) attached to a plastic cup, collecting the organisms in the cup.

2.6.3 Overview of sampling equipment

An overview of sampling equipment, containers used and sampled volumes are shown in

Table 4

Table 4 Equipment and containers used for sampling and necessary sample volume for the individual parameters

Parameter	Collecting devise	Collected volume WST, CT2	Collected volume TT1, TT2
Turbidity	Measured directly	-	-
pH	Measured directly	-	-
Temperature	Measured directly	-	-
Salinity	Measured directly		
Dissolved oxygen	Measured directly	-	-
DOC**	Clean plastic bottle	3x 1000 ml	3x 1000 ml
POC**			
TSS			
UV-trans			
Disinfection by-products analysis (DBP)	Ignited glass bottle with thiosulfate (150 mg/l)	6x 1000 ml	6x 1000 ml 1x 2500 ml***
Total residual oxidants (TRO)	Clean glass bottle pretreated with bleach to remove any chlorine demanding substances	1x 1000 ml	1x 1000 ml
Sludge characterization	Clean plastic bottle	-	1x 2000 ml
Organisms ≥ 50 µm	50 µm sieve with a plastic collection cup. Transferred to a clean glass bottle	3x 20 – 100 l	3x 1 m^3
Organisms 10-50 µm	Clean glass bottle, clean plastic bucket	3x 1000 ml	3x 10l
Heterotrophic bacteria	Sterile bottle with thiosulfate (150 mg/l)	3x 1000 ml (6x500 ml)	3x 1000 ml (6x500 ml)
Coliform bacteria, *E. coli*			
Enterococcus group bacteria			
Vibrio spp.			
Vibrio cholerae			
Acute/Chronic Algae, Copepods, rotatoria, oyster embryo	Clean glass bottle	1000 ml	1000 ml
Acute fish, Juvenile fish	Stainless steel container	300 l	300 l

* A 20-100-litre grab sample (from influent and control water) or 1 m^3 collected through a siphon spillway (from treated water) is concentrated to a volume of 40-100 ml through a plankton net with diagonal dimensions of 50 µm.

** When arriving at the laboratory at NIVA the portion of the water to be analysed for dissolved and particulate organic carbon (DOC and POC, respectively) was transferred to 100 ml acid washed glass bottles before preservation with sulphuric acid (see **Table 5**).

*** An additional top-filled 2.5 litre ignited glass bottle was collected and stored at 4ºC in the dark at Solbergstrand as a spare sample for each collected sample.

2.6.4 Sample preservation and transportation

The international guidelines for preservation and handling of water samples, as described in NS-ISO 5667-3 (2003) and EN ISO 19458 (2006), was followed. All samples, which had to been sent to a laboratory for analysis, were collected, clearly marked, stored in a cooler bag (4°C) and transport to the lab at the end of the day. When the samples arrived at the laboratory, they were stored in a cool room. The samples for organic carbon measurements were preserved with 1ml H_2SO_4 per 100 ml of sample (pH<2) and cooled at 4°C until they were analysed. The samples for disinfection by-products measurements were preserved with thiosulfate (150 mg per 100 ml sample). All details regarding arrivals, storage and analysis of samples were decleared with the sub-contractor laboratories prior to commencing each test cycle. Details regarding sample preservation and storage times before analysis are shown in **Table 5**.

Table 5 Sample preservation and maximum recommended and actual storage times before analysis

Parameter	**Preservation**	**Maximum recommended holding time**	**Actual storage time**
Temperature	-	In situ	In situ
pH	-	In situ	In situ
Dissolved oxygen	-	In situ	In situ
Salinity	-	-	In situ
Turbidity	-	24h	In situ (probe) 0-24 hours (lab)
Total Residual Oxidants (TRO)	-	10 min	<10 min
Disinfection by-products (DBP)	Neutralisation with thiosulfate. Stored in dark, 4°C, top-filled bottles	7 days	0-7 days
Dissolved organic carbon (DOC)	Acidify with 1 ml 4 M H_2SO_4 per 100 ml (pH<2), 4°C	7 days	0-5 days
Total organic carbon (TOC)	Acidify with 1 ml 4 M H_2SO_4 per 100 ml(pH<2), 4°C	7 days	0-5 days
Particulate organic carbon (POC)	Acidify with 1 ml 4 M H_2SO_4 per 100 ml(pH<2), 4°C	7 days	0-5 days
Total suspended solids (TSS)	4°C	24 hours	<24 hours
Ultraviolet transmission (UV)	4°C	24 hours	<24 hours
Organisms ≥ 50 µm	4°C	6 hours	< 2 hours
Organisms 10-50 µm	4°C	24 hours	< 24 hours
Heterotrophic bacteria	With thiosulfate, 4°C	24 hours	< 24 hours
Coliform bacteria, *E. coli*			
Enterococcus group bacteria			
Vibrio spp.			
Vibrio cholerae			
Acute/Chronic Algae, Copepods, rotatoria, oyster embryo	4°C	24 hours	< 24 hours
Acute fish, Juvenile fish	16°C	24 hours	< 24 hours

2.7 *In situ* measurements

pH

pH was measured *in situ* using a calibrated probe and pH-meter (WTW pH/cond 340i, back-up WTW pH 340).

Dissolved oxygen (DO)

Dissolved oxygen (DO) was measured *in situ* using a calibrated probe and meter (WTW oxy 330i). DO is reported as mg O_2/l.

Salinity

Salinity is measured *in situ* using a calibrated salinoterm (WTW cond 330i, WTW pH/cond 340i). Salinity is reported in PSU.

Temperature

Temperature was measured *in situ* using the built-in thermometers in the oxygen probe (WTW oxy 330i) and in the salinoterm (WTW pH/cond 340i) and calculated as the average of the two readings. Temperature is reported in ºC.

2.8 Analyses of discrete samples

2.8.1 Chemical analyses

Total residual oxidants (TRO)

Total residual oxidants (TRO) was measured by the colorimetric DPD-method (American Public Health Association, 1989), which is currently the method recommended for measurement of TRO in seawater (Buchan et al., 2005). The method is based on the oxidation of N,N-diethyl-p-phenylendiamin (DPD) which turns to a pink Wurster-cation in the presence of strong oxidants. The intensity of the colour is proportional to the TRO concentration. The colour intensity is measured by a Hach DR/2000 spectrophotometer (Hach Company, Loveland, CO, USA). The method and the instrument give the results as total residual oxidants (TRO) as mg/l Cl_2. TRO are reported as free and total concentration of chlorine. The detection range of this method is 0.02-2.0 mg/l.

Turbidity

Turbidity was measured using a 6035 Turbidimeter (Jenway) with formazin as standard (Formazin Turbidity Standard 4000 NTU, HACH, 2461-42 and reported as Formazin Nephelometric Units (FNU) or as Nephelometric Turbidity Units (NTU).

Dissolved and total organic carbon (DOC and TOC)

DOC and TOC were measured by accredited methods based on Norwegian Standard NS-ISO 8245 (NIVA method G5-3) at NIVA: TOC is measured on the whole sample and DOC is measured after filtering the sample through a GF/F filter (0.7 µm). The sample is acidified with phosphoric sulfuric acid and aerated with oxygen to remove inorganic carbon. The sample is injected in a quartz tube filled with a platinum catalyser at 680 ^oC. The organic carbon compounds are oxidized to CO_2 which is quantified using an NDIR detector (Phoenix 8000 TOC-TC analyser with sample carousel STS 8000) with oxygen as carrier. Detection limit is 0.2 mg C/l.

Particulate organic carbon (POC)

POC was calculated as the difference between the level of TOC in the sample, measured on the non-filtered sample, and the measured DOC level of the same sample. POC was also measured as the amount of organic matter accumulating on a glass fibre filter GF/F (0.7 µm) when a known amount of sample is filtered (NIVA method G6): The dry sample is encapsulated in tin capsules which are ignited in oxygen saturated heliumgas at 1800 ºC. Surplus oxygen is removed by Cu at 650 ºC and the off-gases are passed through a chromatographic column, where upon CO_2 is detected (Thermo Flash 2000 element analyzer). The method is based on CARLO ERBA ISTRUMENTAZIONE, ELEMENTAL ANALYZER 1106. Instruction manual, APPLICATION LAB REPORTS, Elemental analysis lab, Carlo Erba. January 1987. Detection limit is depending of the volume of sample filtered. For 50-100ml filtered sample, the detection limit is between 0.05 and 0.1 mg C/l.

Total suspended solids (TSS) and organic content

TSS was measured using NIVA method B1/2 in accordance with NS-EN 872 and NS 4733: A glassfiber filter GF/F (0.7 µm) is prepared by washing it with distilled water and drying at 105 ^{o}C for 30 minutes followed by two hours at 480 ^{o}C, cooling and final weighing. The sample is filtered through the filter.The filter is dried for 1 hour and weighed. The TSS is represented by the weight increase. Lowest reported value is 0.1 mg/l. The filter with the dry residues is then ignited at 480 ^{o}C for two hours, cooled down and weighed. The loss on ignition is reported as the organic content of the TSS.

Characteristics of sludge from filter backflushing

In one test cycle for each test water quality the backflush water from the filter was analysed. The following parameters were measured: pH, salinity, dissolved oxygen, temperature, turbidity, TOC, DOC, POC, TSS and organic content of TSS (ignition loss), settling solids and their density.

The methods used to determine pH, salinity, dissolve oxygen, temperature, turbidity, TOC, POC, DOC, TSS and organic content of TSS in the sludge samples were the same methods as used for the other water samples as described in this chapter 2.

Settling solid was measured at NIVA in accordance with NS-EN 14702-1:2006 Characterisation of sludges - Settling properties - Part 1: Determination of settleability (Determination of the proportion of sludge volume and sludge volume index). But because of the low total amounts of suspended material in the samples, determination of settleability was replaced by determination of total dry matter (TDM). The method used was according to the method EN 12880 (see paragraph 7.2.1 in NS EN 14702-1). The principle of the measurement was to evaporate 250 ml of the sludge sample at 105°C during one day or more until completely dryness which was considered to be reached when the weight became constant with time.

Density was determined at NIVA as the weight of a known volume of the sample, divided by the volume of the sample.

Disinfection by-products

Table 6 lists the disinfection by-products (DBPs) analysed by external laboratories. All DBPs were analysed in both influent (WST), control and treated water on day 0 and day 5 in the two first test cycles for each water quality, except for the control water samples which was analysed only in the first test cycle for each water quality.

Table 6 Disinfection by-products included in the analyses

Disinfection by-products	**Unit**	**Detection Limit**	**Laboratory**
Trichloromethane (chloroform)	µg/l	0.1	ALS
Bromodichloromethane	µg/l	0.1	ALS
Dibromochlorormetane	µg/l	0.1	ALS
Tribromomethane (bromoform)	µg/l	0.1	ALS
Chloroacetic acid (MCAA)	µg/l	0.5	ALS
Dichloroacetic acid (DCAA)	µg/l	0.3	ALS
Trichloroacetic acid (TCAA)	µg/l	0.2	ALS
Bromoacetic acid (MBAA)	µg/l	0.2	ALS
Dibromoacetic acid (DBAA)	µg/l	0.1	ALS
Bromochloroacetic acid (BCAA)	µg/l	0.1	ALS
Dichlorobromoacetic acid (DCBAA)	µg/l	0.1	ALS
Dibromochloroacetic acid (DBCAA)	µg/l	0.1	ALS
Tribromoacetic acid (TBAA)	µg/l	0.1	ALS
Dichloroacetonitrile	µg/l	0.1	ALS
Trichloroacetonitrile	µg/l	0.1	ALS
2,4-Dibromophenol	µg/l	0.1	ALS
2,6-Dibromophenol	µg/l	0.1	ALS
2,4,6-Tribromophenol	µg/l	0.1	ALS
1,2-Dibromoethane	µg/l	0.1	ALS
1,2,4-Tribromobenzene	µg/l	1.0	ALS
1,2,3-Trichloropropane	µg/l	0.1	ALS
2-Chlorotoluene	µg/l	0.1	ALS
4-Chlorotoluene	µg/l	0.1	ALS
1,2-Dibromo-3-chloropropane	µg/l	0.1	ALS
1,2,3-Tribromobenzene	µg/l	1.0	ALS
1,3,5-Tribromobenzene	µg/l	1.0	ALS
Monobromoacetonitrile	µg/l	0.1	ALS
Dibromoacetonitrile	µg/l	0.1	ALS
Bromochloroacetonitrile	µg/l	0.1	ALS
Dibromomethane	µg/l	0.1	ALS
AOX	mg/l	0.020	ALS
EOX	mg/l	0.010	ALS
Bromate	µg/l	1.0	DVGW

Adsorbable organically bound halogens (AOX)

Determination of adsorbable organically bound halogens (AOX) was performed as described in DIN EN ISO 9562:2004 by a solid phase extraction (SPE) in waters with high salt content. AOX represents the sum of organically bound chlorine, bromine and iodine (but not fluorine) which can be adsorbed on activated carbon under specified conditions and, if the sample is not filtered, includes that associated with suspended matter.

Extractable organically bound halogens (EOX)

Extractable organically bound halogens (EOX) was measured by solvent extraction and microcolorimetric method as described in DIN 38409-H8:1984.

Trihalomethane compounds (THMs)

Trichloromethane (chloroform), bromodichloromethane, dibromochloromethane, tribromomethane (bromoform) were analysed by the purge and trap method described in DIN EN ISO 15680/ US-EPA 524.2 with a GC-MS detection.

Bromate

Bromate ion was measured by Liquid Ion Chromatography as described in DIN EN ISO 10304-1:1992 Determination of dissolved fluoride, chloride, nitrite, orthophosphate, bromide, nitrate and sulfate ions, using liquid chromatography of ions. Part 1: Method for water with low contamination.

Haloacetic acids (HAA)

Haloacetic acids (HAA) were determined by gas chromatography (GC-MS detection) after a liquid-liquid extraction and a derivatization as described in EN ISO 23631/DIN 38407 F25.

Halogenated acetonitrile compounds

Monobromoacetonitrile, dibromoacetonitrile, bromochloroacetonitrile, dichloroacetonitrile and trichloroacetonitrile were analysed by the American US EPA 551.1 method: determination of chlorination disinfection by-products, chlorinated solvents, and halogenated pesticides/herbicides in drinking water by liquid-liquid extraction, derivatisation and gas chromatography with electron-capture detection.

Bromophenol

Bromophenol compounds were measured by gas chromatography with mass spectrometry detection after a liquid-liquid extraction and derivatisation.

Tribromobenzen, chlorotoluene and halogenated aliphates

Tribromobenzene, chlorotoluene and the halogenated aliphates were analysed according to DIN EN ISO 10301-F4/ US EPA 524.2: purge and trap gas chromatography with a mass spectrometry detector.

2.8.2 Identification and quantification of organisms

Identification and quantification of organisms ≥50 µm

Samples collected for organisms >50 µm were inspected using a stereo loupe at 10-40x magnification within 6 hours after sampling. Viable organisms were counted and identified based on motility and integrity according to OECD (1985): OECD Test Guideline for Testing of Chemicals 202, "*Daphnia* sp. acute immobilisation test and reproduction test". Since it may take several hours for the lethal actions of the UV irradiation to show effect on motility of the exposed organisms, treated samples with observable live organisms were always recounted after 24 hours both for samples on day 0 and day 5.

Identification and quantification of organisms ≥10-50 µm

The viability of the micro-plankton (≥10-50 µm) was determined by observing cells incubated with 5-carboxyfluorescein diacetate acetoxymethyl ester (CFDA-AM) according to Ganassin et al. (2000). A 10 ml sample was incubated for 1 hour with 4 µmol of CFDA-AM. The sample was fixed with formalin and filtered onto black polycarbonate filters. The filter was mounted on a glass slide in paraffin oil and frozen. CFDA-AM is hydrolysed only in living cells. CFDA-AM is a marker for cell membrane integrity and may be measured directly in cells. In principle, the non-fluorescent chemicals CFDA-AM is taken up in the cytosol, where it becomes hydrolysed into fluorescence end products. These end products are trapped inside the cellular compartment and may be observed in an epifluorescence microscope using excitation filter 485 nm and emission filter of 530 nm. In the epifluorescence microscope viable cells are observable as brightly yellow/green coloured cells, while non viable cells are pale green (heterotrophic cells) or pale green with red autofluorescence of the chloroplast (photoautotrophs). Numbers of viable and non viable cells were counted at 300x – 480x magnification. Since it may take several hours for the leathal actions of the UV irradiation to show effect on cell viability of exposed organisms, treated samples with observable live organisms were always performed 24 hours after the UV exposure. During those 24 hours the samples were stored at $4^{o}C$ in the dark.

As a complementary method to direct counting for testing of viability, the serial dilution method in algal growth medium was used. The serial dilution method is often referred to as the most probable number method. It is simply based on the fact that by diluting the sample in a sequence and observing in which dilutions the organisms occur (grow) the following two weeks, one is able to backward calculate the number of cells in the original sample. The dilution series were achieved by adding 1 ml of sample to 9 ml of media (algal growth media, 20 % Z8 seawater media). After mixing, 1 ml of this sample was further diluted with 9 ml. In this way a series of 10x dilution were made. The number of dilutions was set to cover the expected cell density range in the original sample. 3-5 parallels were employed in order to provide statistical significance of estimated number.

A supplementary cultivation test was also used by plating on agar plates. 100 µl of samples was spread on a seawater agar growth medium and incubated in constant light for 72 hours at 20 ^{o}C. Colonies of *Tetraselmis sp.* was observed by viewing agar plates in stereo microscope at 160x magnification. The procedure has a detection limit of 10 cells/ml, and was used as a rapid estimation of viable *Tetraselmis sp.* in the samples.

Identification and quantification of bacteria

The samples were diluted or concentrated to achieve a quantifiable concentration of colony forming units on a solid growth media (agar-medium plates) or a medium-amended filter paper. The dilution or concentration was based on experience and expectance of the concentration of the bacteria in the sample. Dilution was performed by stepwise 10x dilution of the sample in a dilution series followed by incubation on agar. Concentration was performed by filtering a predetermined sample volume through a sterile filter followed by incubation of the filter on growth media.

Heterotrophic bacteria

Heterotrophic bacteria were quantified according to a modified version of Norwegian Standard NS-EN 6222:1999 using a marine agar for isolation of marine heterotrophic bacteria at a temperature of 22±1°C and an incubation period of 3±1 days.

Coliforms

Coliform bacteria were quantified according to Norwegian Standard NS 4788 at a temperature of 37±1°C and an incubation period of 22-24 hours.

E.coli

E. coli were quantified according to Norwegian Standard NS 4792 or NS-EN ISO 9308-3 at a temperature of 44.5±0.2 °C and an incubation period of 18-24 hours.

Enterococcus group

Total fecal *Enterococci* were quantified according to Norwegian Standard NS-EN ISO 7899-2 at a temperature of 36±2 °C and an incubation period of 44 hours.

Intestinal *Enterococci*
Intestinal *Enterococci* were confirmed according to Norwegian Standard NS-EN ISO 7899-2 at a temperature of 44±0.2 °C and an incubation period of 2 hours.

Vibrio species *and Vibrio cholerae*
The total number of *Vibrio* spp., were determined by filtration of 1-100 ml sample, and by placing the filter on TCBS Cholera-medium agar plates (CMO333 from Oxoid). Plates were incubated at 37 °C, and colonies counted after 24 hours incubation. The TCBS Cholera-medium supports the growth of pathogenic Vibrios (e. g. *Vibrio cholerae*, *Vibrio parahaemolyticus*) as well as some other Vibrios and other bacterial species, i.e. *Aeromonas hydrophila.*

The strategy for elimination or identification of serotypes O1 and O139 were as follows:
The morphology of the colonies developing on the TCBS-medium after 24 h was visually studied. Colonies with distinct colour and morphology different from *Vibrio cholera* were not selected for further identification. Colonies with typical *Vibrio cholera* appearance were re-striked on TCBS medium and again inspected for growth and morphology. Classical elimination or identification methods were used, such as appearance in culture media and physiological and biochemical properties. If *Vibrio cholera* was identified, polymerase chain reaction (PCR) would be used for elimination or identification of the serotypes O1 and O139.

For samples with high number of *Vibrio spp.*, we use an optional method which is a modification of the method described by Huq *et al*. (2006). It is based on enrichment in alkaline peptone water (APW), followed by culturing of surface growth from APW on TCBS, and sub-culturing on nutrient agar without NaCl. It is a presence-absence method. The method should be performed for samples with high numbers of presumptive *Vibrio* spp. (>100 per 100 ml) where we have to confirm absence of *V. cholerae* in 100 ml water sample.

2.8.3 Toxicity measurements of treated ballast water

The following standard tests were performed on the treated ballast water.

- Growth inhibition of the marine alga *Skeletonema costatum* according to ISO 10253: Marine algal growth inhibition test.
- Acute toxicity to the marine crustacean *Acartia tonsa* according to ISO 14669: Determination of acute lethal toxicity to marine copepods (*Copepoda, Crustacea*).
- Reproductive toxicity to the marine crustacean *Nitocra spinipes* according to “Forslag til Dansk Standard: Økotoksikologisk undersøgelse med krepsdyret *Nitocra spinipes*. Reproduksjonsmetode”.
- The oyster embryo bioassay according to the ASTM method (E724) and the comprehensive guidelines laid out in the ecotoxicity test methods for effluent and receiving water assessment (EA, 2001).
- Acute toxicity to juvenile turbot (*Scopthalmus maximus*) according to OECD Guidelines for testing of chemicals (No. 203; Fish, acute toxicity test).
- Chronic toxicity to juvenile turbot (*Scopthalmus maximus*) according to OECD Guidelines for testing of chemicals" (No. 215; Fish, juvenile growth test), adapted for marine species.
- Chronic toxicity using rotatoria reproduction test with the marine species *Brachionus plicatilis* based on a standard test developed for the related freshwater species *Brachionus calyciflorus* (ISO 20666 – Determination of the chronic toxicity to *Brachionus calyciflorus*).

Growth inhibition of the marine alga *Skeletonema costatum*
The inhibitory effect of treated ballast water on the growth of the marine diatom *Skeletonema costatum*, strain NIVA BAC1, has been investigated. The test was performed according to ISO 10253: Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum.*

A concentration series of treated ballast water diluted in untreated water was prepared. The batches were inoculated with test algae and incubated on a shaking table at 20 ± 2 °C, under continuous illumination. Growth was monitored by daily counting of cell numbers using a Coulter Multisizer. The tests were performed with three replicates at each concentration and six control replicates in untreated ballast water.

The growth rate in each culture was calculated from the increase in cell density during three days exposure. Growth rates were calculated as percentage of growth rate in the controls (untreated ballast water) and plotted against concentration of treated ballast water. From the respons plot, the concentrations causing 10% and 50 % inhibition of the growth rate (i.e. EC_{10} and EC_{50}) were derived by non-linear regression analysis.

Reproductive toxicity to the marine crustacean *Nitocra spinipes*
The reproductive toxicity of treated ballast water to the marine crustacean *Nitocra spinipes* has been investigated. The test was performed according to Draft guideline for Danish Standard: ”Økotoksikologisk undersøgelse med krepsdyret *Nitocra spinipes*. Reproduksjonsmetode”. Test was performed as a limit test as defined in "OECD Guidelines for testing of chemicals" (No. 203; Fish, acute toxicity test), with one test concentration of 100 % treated ballast water and using non treated ballast water as control water.

The test was performed with 20 replicate vessels with 1 pregnant female in each vessel. The vessels were incubated for 14 days at 20 °C. The total number of living offspring was counted.

Acute toxicity to the marine crustacean *Acartia tonsa*
The acute toxicity of treated ballast water to the marine crustacean *Acartia tonsa* has been investigated. The test was performed according to ISO 14669: Determination of acute lethal toxicity to marine copepods (*Copepoda, Crustacea*). The test concentrations were in the range 32 to 100 % of treated ballast water.

The test was performed with four replicate vessels with 4-8 test animals for each test concentration and sixteen control replicate vessels. The vessels were incubated for 48 hours at 20 ± 1 °C. Mortality was recorded after 24 and 48 hours.

Chronic toxicity to juvenile turbot (*Scopthalmus maximus*)
The chronic toxicity of treated ballast water to turbot was tested in accordance with the "OECD Guidelines for testing of chemicals" (No. 215; Fish, juvenile growth test), adapted for marine fish (McWilliams, 1994).

The testing of chronic toxicity of treated ballast water is required by the IMO G9 guidelines for testing of treatment technology. The fish was exposed continuously for 28 days with water exchange 3 times per week. Test was performed as a limit test as defined in "OECD Guidelines for testing of chemicals" (No. 203; Fish, acute toxicity test), using one test concentration of 100 % treated ballast water and using non treated ballast water as control water. Weight of each fish was recorded at start and at end, and a specific growth rate was estimated.

The test water was taken directly from control and test tank (TT2 and TT1) (100% ballast water), after conditioning to the test temperature.

Acute toxicity towards the juvenile turbot (*Scopthalmus maximus*)
The acute toxicity of treated ballast water towards turbot was tested in accordance with the draft procedure of McWilliams (1994). The procedure follows the general guidelines of OECD 203 "Fish, Acute toxicity test".

The testing of acute toxicity of treated ballast water is required by the IMO G9 guidelines for testing of treatment technology. The fish was exposed continuously for 96 hours with full water exchange every day. Test was performed as a limit test as defined in "OECD Guidelines for testing of chemicals" (No. 203; Fish, acute toxicity test), using one test concentration of 100 % treated ballast water and using non treated ballast water as control water.

10 juvenile turbot were used in each aquarium with 40 l of medium. The test water was taken directly from the control and test tank (TT2 and TT1) (100% ballast water), after conditioning to test temperature.

The oyster embryo bioassay

The oyster embryo bioassay (OEB) is based on the ASTM method (E724) and the comprehensive guidelines laid out in the ecotoxicity test methods for effluent and receiving water assessment (EA, 2001). The OEB is a sensitive *in vivo* test that measures the response of the most sensitive life stage of the oyster to contaminant exposure. The bioassay measures the success of trocophore larvae to develop into a normal D-stage veliger larvae following 48 hour exposure to test media. The frequency of normal D-stage larvae following 48 hour exposure is determined microscopically to provide an assessment of sample toxicity. The data generated enables standard toxicity values such as EC_{10}, EC_{50} and NOEC (no observable effect concentration) and LOEC (lowest observable effects concentration) values to be determined for the test sample.

Rotatoria reproduction test

The chronic toxicity to rotatoria was studied using the marine species *Brachionus plicatilis*. The rotifera were kept in a laboratory culture fed with live algae. The test procedure is based on a standard test developed for the related freshwater species *Brachionus calyciflorus* (ISO 20666 – Determination of the chronic toxicity to *Brachionus calyciflorus*). Briefly, freshly hatched rotatoria were incubated individually in a series of concentrations of the test water and in control water for 72 hours. At the end of the test, the number of egg and offspring were determined and compared with the control, i.e. the non treated ballast water. The population growth percentages were determined for each concentration of the test water.

3. Results and discussion

3.1 Fullfilment of the initial chemical water quality test criteria

The required chemical water quality of the test water, as stated in G8 and shown in **Table 7** for medium salinity test cycles (1-5) and **Table 8** for high salinity test cycles (6-11), was considered as fulfilled for all test cycles. In test cycle 3, the average values for particulate organic carbon (POC) and total suspended solids (TSS) were marginally below the accepted minimum levels. However, the values are within the precision of the methods given as standard deviations. The DOC, POC and TSS concentrations show little variation between the test cycles; <0.5 mg/l for DOC, <1.0 mg/l for POC and <7 mg/l for TSS for each water quality. The average concentrations in brackish water were 5.2 mg/l for DOC, 5.3 mg/l for and 51.8 mg/l for TSS. Average concentrations in seawater were 2.2 mg/l for DOC, 3.0 mg/l for POC and 16.2 mg/l for TSS. Measurements of UV transmission (UV-T) in the test water quality in the WST tank showed variations between 62% and 66% for the brackish water test cycles and between 88% and 93% for the seawater test cycles. Turbidity measured in the WST tank before sampling and treatment is shown in Appendix 1 (Table 2). The low variation of the turbidity measurements, under 5%, confirmed the homogeneity of the test water in each tank before sampling. The numbers shown in **Table 7** and **Table 8** are average values of three samples and the standard deviation of the mean.

Table 7 Concentrations of dissolved organic carbon (DOC), particulate organic carbon (POC), total suspended solids (TSS) and salinity and UV-transmission (UV-T) in the influent water (WST) before treatment for medium salinity test cycles (1-5)

	Salinity	**DOC**	**POC**	**TSS**	**UV-T**
	[PSU]	**[mg C/l]**	**[mg C/l]**	**[mg/l]**	**%**
Requirement	3-22	>5	>5	>50	-
Test cycle 1	21.2	5.5 ± 0.3	5.6 ± 0.4	50.7 ± 0.9	66.0 ± 0.1
Test cycle 2	21.4	5.0 ± 0.1	5.8 ± 0.1	53.5 ± 1.6	64.4 ± 0.8
Test cycle 3	21.3	5.2 ± 0.1	4.8* ± 0.2	49.7 ± 1.0	65.1 ± 0.5
Test cycle 4	21.7	5.4 ± 0.1	5.1 ± 0.6	51.1 ± 1.6	65.0 ± 0.3
Test cycle 5	21.2	5.0 ± 0.1	5.3 ± 0.5	54.1 ± 0.2	62.1 ± 0.6

* By using the other POC calculation method, as desbribed in 2.8.1, (i.e. by calculation of the difference between the level of TOC in the sample, measured on the non-filtered sample, and the measured DOC level of the same sample), the POC value is 5.3 ± 0.2 mg/l. The average of the results of the two analysis methods is > 5 mg/l.

Table 8 Concentrations of dissolved organic carbon (DOC), particulate organic carbon (POC), total suspended solids (TSS) and salinity and UV-transmission (UV-T) in the influent water (WST) before treatment for high salinity test cycles (6-11)

	Salinity	**DOC**	**POC**	**TSS**	**UV-T**
	[PSU]	**[mg C/l]**	**[mg C/l]**	**[mg/l]**	**%**
Requirement	>32	>1	>1	>1	-
Test cycle 6	32.1	1.9 ± 0.1	2.8 ± 0.1	14.2 ± 2.1	93.2 ± 0.7
Test cycle 7	32.2	2.0 ± 0.1	2.8 ± 0.1	14.8 ± 4.3	92.4 ± 0.2
Test cycle 8	32.3	2.1 ± 0.1	2.7 ± 0.1	13.0 ± 0.3	89.8 ± 0.7
Test cycle 9	32.6	2.1 ± 0.1	2.8 ± 0.2	18.3 ± 3.3	90.9 ± 0.1
Test cycle 10	32.1	2.2 ± 0.1	3.7 ± 0.1	19.8 ± 3.7	88.1 ± 0.6
Test cycle 11	32.3	2.8 ± 0.1	3.0 ± 0.1	17.0 ± 4.6	88.7 ± 0.2

3.2 Fulfillment of the initial biological water quality test criteria

3.2.1 Organisms ≥50 µm in minimum diameter

The initial concentration of organisms ≥50 µm in minimum diameter in the WST tank before commencing the test cycles (day 0) are shown in **Table 9**. The requirements, as stated in G8 and shown in the table, were met in all test cycles. Species variability was determined as described in section 2.4.2. and 2.6.2 in samples collected from the storage tank for harvested organisms, and from the WST tank.

Table 9 Fulfilment of biological water quality test criteria regarding organisms ≥50 µm in minimum diameter. Green background indicates fulfilment, yellow background indicates partial fulfilment and red background indicates lack of fulfilment

	Microscope counts	**Phyla***	**Species***
	[organisms/m^3]	**[-]**	**[-]**
Requirements	≥100 000	≥3 different	≥5 different
Test cycle 1	212 300 ± 18 631	6	15
Test cycle 2	180 900 ± 6 062	6	11
Test cycle 3	184 294 ± 22 623	6	11
Test cycle 4	150 479 ± 25 755	6	13
Test cycle 5	179 400 ± 21 826	5	11
Test cycle 6	156 000 ± 17 920	5	11
Test cycle 7	144 013 ± 14 258	5	12
Test cycle 8	183 467 ± 9 626	6	17
Test cycle 9	177 425 ± 5 073	5	12
Test cycle 10	182 042 ± 21 651	5	13
Test cycle 11	165 263 ± 14 250	5	14

* Numbers referring to all phyla and species observed in pipeline from harvesting tank and WST

3.2.2 Organisms ≥10-50 µm in minimum diameter

The initial concentration of organisms ≥10-50 µm in minimum diameter in the WST tank before commencing the test cycles (day 0) are shown in **Table 10**. The requirements, as stated in G8 and shown in the table, were met in all test cycles with all three detection methods. Species diversity was determined in samples taken from the storage tank for harvested organisms, and samples from the WST tank. The CFDA-AM staining method was used to determine viability (see section 2.4.2 and 2.4.6).

Table 10 Fulfilment of biological water quality test criteria regarding organisms >10-50 µm in minimum diameter. Green background indicates fulfilment, yellow background indicates partial fulfilment and red background indicates lack of fulfilment

	Dilution method		**Microscope counts**	**Plate counts**	**Phyla***	**Species***
	[cells/ml]	**95 % conf. int.**	**[cells/ml]**	**[cells/ml]**	**[-]**	**[-]**
Requirements	≥1000		≥1000	≥1000	≥3 diff.	≥5 diff.
Test cycle 1	3000	1000-13000	1772 ± 123	1590 ± 215	3	19
Test cycle 2	3000	1000-13000	1340 ± 131	1983 ± 215	3	21
Test cycle 3	3000	1000-13000	2083 ± 54	2043 ± 95	3	17
Test cycle 4	1600	600-5300	1979 ± 196	1433 ± 40	4	21
Test cycle 5	1400	600-3600	2541 ± 263	1927 ± 157	4	18
Test cycle 6	2400	1000-9500	1219 ± 158	1167 ± 78	4	16
Test cycle 7	2400	1000-9500	1806 ± 261	1920 ± 265	4	16
Test cycle 8	1600	600-5300	1383 ± 227	1580 ± 72	4	21
Test cycle 9	1600	600-5300	1158 ± 65	1327 ± 144	4	18
Test cycle 10	3000	1000-13000	2100 ± 69	1843 ± 92	4	24
Test cycle 11	5000	2000-20000	2014 ± 338	1957 ± 58	4	18

*Numbers referring to all phyla and species observed in pipeline from harvesting tank and WST

3.2.3 Heterotrophic bacteria

G8 requires that the heterotrophic bacteria count should be ≥10^4 cfu/ml. As shown in **Table 11**, this requirement was fulfilled in all test cycles**.**

The IMO G8 guideline 2.3.20 also specifies that "…the following bacteria do not need to be added to the influent water, but should be measured at the influent and at the time of discharge: 1. Coliform; 2. Enterococcus group; 3. *Vibrio cholerae* and 4. Heterotrophic bacteria." Initial concentrations of these bacteria groups are shown in **Table 11.**

Table 11 Fulfilment of biological water quality test criteria regarding microorganisms. Green background indicates fulfilment, yellow background indicates partial fulfilment and red background indicates lack of fulfilment

	Marine heterotrophic bacteria	**Coliform bacteria**	**Vibrio spp.**	**Enterococcus group**
	[cfu/ml]	**[cfu/ml]**	**[cfu/100 ml]**	**[cfu/100 ml]**
Requirements	≥10^4	-	-	-
Test cycle 1	1.3 ± 0.7 x10^4	<1	1.4 ± 0.4 x 10^4	1.5 ± 0.7
Test cycle 2	1.0 ± 0.07 x10^4	<1	5.5 ± 0.04 x 10^4	2.7 ± 0.6
Test cycle 3	1.0 ± 0.9 x10^4	<1	2.7 ± 1.9 x 10^2	<1
Test cycle 4	1.3 ± 0.3 x10^4	<1	4.9 ± 2.3 x10^2	<1
Test cycle 5	2.1 ± 0.2 x10^4	<1	9.9 ± 4.3 x10^3	<1
Test cycle 6	1.4 ± 0.2 x10^4	<1	1.2 ± 0.1 x10^4	<1
Test cycle 7	1.3 ± 0.3 x10^4	<1	1.1 ± 0.1 x10^3	<1
Test cycle 8	1.6 ± 0.1 x10^4	<1	1.4 ± 0.02 x10^3	<1
Test cycle 9	1.1 ± 0.2 x10^4	<1	2.6 ± 0.5 x10^2	<1
Test cycle 10	1.9 ± 0.1 x10^4	<1	9.2 ± 0.4 x10^2	<1
Test cycle 11	1.6 ± 0.2 x10^4	<1	5.9 ± 1.9 x10^1	<1

3.3 Operational performance of the Auramarine Crystal Ballast BWMS

A total of 11 test cycles were completed in the period from January 20 to April 12 in 2010. Each test cycle consisted of ballasting, five days storage and consecutive deballasting. For the treatment cycle the test water was treated both during ballasting (filtration and UV irradiation) and during deballasting (UV irradiation only), while for the control cycle the test water was only transferred between tanks using the same pump as was used for the water transfers during the treatment cycle. Test cycles 1-5 were conducted with brackish water (21.2-21.7 PSU) and test cycles 6-11 with high salinity seawater (32.1-32.6 PSU).

The operational performance of the Auramarine Crystal Ballast BWMS (ACB-BWMS) was monitored by the site responsible person from NIVA during each test cycle; water flow rates were both measured and calculated (**Table 12**), the duration of ballasting and deballasting for treatment and control cycles was measured (**Table 12**), the UV intensities in the UV chamber were measured and read from the control panel of the ACB-BWMS during treatment (**Table 13**). The reports from these controls are shown in **Appendix D**.

Shortly summarised:

- Water flow and test duration: Measured average flow rate during ballasting and deballasting for all treatment runs and control runs were 251-252 m^3/h with a very low variability (± 1-2 m^3/h) (see **Table 12**). The calculated flow rates (from the total volume pumped and the duration of pumping) showed somewhat higher variability (± 5-10 m^3/h), but the average flow rates for all tests were 251-252 m^3/h (**Table 12**). Ballasting operations lastet 53-55 minutes, while deballasting operations lasted 47-51 minutes (**Table 12**) (partly due to reduced water volumes caused by sampling in the meantime and water volumes left in the pipes and bottom of the tanks after each operation).
- UV intensities: For the cycles with brackish water the measured average UV intensities were between 121 and 142 mW/cm^2 during ballasting (test cycle 1-5) and between 115 and 148 mW/cm^2 during deballasting (test cycle 1-4). See **Table 13**. It was observed that UV-intensity varied somewhat with the time since last sensor-cleaning. For the seawater test cycles the UV intensities were between 203 and 267 mW/cm^2 during ballasting and between 179 and 260 mW/cm^2 during deballasting (**Table 13**). As expected, a somewhat higher UV intensity was measured during UV irradiation of seawater than of brackish water due to the higher initial level of supended solid and DOC in brackish water (compare **Table 7** and **Table 8**). For test cycle 11 the average UV intensity was reduced to 81 mW/cm^2 during ballasting and to 91 mW/cm^2 during deballasting, or 34 % and 41 %, respectively, of the average UV intensities during test cycles 6-10 (**Table 13**).
- Energy consumption: The energy input was kept constant during ballasting and deballasting for test cycles 1-10, as shown by the relatively stable energy consumption level from cycle to cycle given in **Table 13**. In cycle 11, when the UV irradiation was reduced, the concomitant energy consumption was reduced to 45-46 % of the average energy consumption during test cycles 6-10.

Table 12 Test duration and water flows during ballasting and deballasting during test treatment with the Auramarine Crystal Ballast BWMS and control for all 11 test cycles

Test cycle	**Date (in 2010)**	**Crystal Ballast treatment**				**Control**			
		Day 0 - ballasting (WST to TT2)		**Day 5 - deballasting (TT2 to TT1)**		**Day 0 - ballasting (WST to CT2)**		**Day 5 - deballasting (CT2 to TT2)**	
		duration	Flow	duration	flow	duration	flow	duration	flow
		[min]	[m^3/h]	[min]	[m^3/h]	[min]	[m^3/h]	[min]	[m^3/h]
1	20.1 - 25.1	55	249	51	251	55	250	54	251
2	27.1 - 1.2	53	251	48	252	54	251	51	251
3	3.2 - 8.2	54	251	48	252	53	251	53	251
4	10.2 - 15.2	53	251	49	252	54	251	51	251
5	17.2 - 22.2	54	251	49	251	53	251	51	251
6	24.2 - 1.3	55	252	50	252	54	251	52	251
7	3.3 - 8.3	55	252	49	252	54	251	50	251
8	10.3 - 15.3	54	251	48	252	55	251	52	251
9	17.3 - 22.3	54	251	48	251	54	251	50	251
10	24.3 - 29.3	55	251	49	251	55	252	51	251
11	7.4 – 12.4	54	251	47	251	55	251	48	252
Average flow rates all tests (m^3/h)									
Measured		251		252		251		251	
Calculated		251		252		252		251	
Flow rate ranges all tests (m^3/h)									
Measured		249-252		251-252		250-251		251-252	
Calculated		245-257		245-257		249-258		240-260	

Table 13 Measured average and range of the intensities delivered by the UV lamps in the UV chamber and the measured energy consumption

Test cycle	Dates (in 2010)	Day 0- ballasting			Day 5 - deballasting			Total
		UV intensity		Energy consum.	UV intensity		Energy consum.	Energy consum.
		Average	Range		Average	Range		
		mW/cm^2	mW/cm^2	kWh	mW/cm^2	mW/cm^2	kWh	kWh
Brackish water test cycles								
1	20.1 - 25.1	121	121-121	19.4	115	115-116	17.5	36.9
2	27.1 - 1.2	142	142-142	19.8	146	131-148	17.2	37.0
3	3.2 - 8.2	136	135-137	18.9	140	136-141	17.3	36.2
4	10.2 - 15.2	131	131-131	19.0	136	119-138	16.9	35.9
5	17.2 - 22.2	131	127-132	18.7	204*	200-205	17.4	36.1
Average ± st.dev. Test cycles 1-5		132 ± 8	-	19.2 ± 0.4	148 ± 33	-	17.3 ± 0.2	36.4 ± 0.5
Seawater test cycles								
6	24.2-1.3	264	260-267	19.1	248	238-260	18.2	37.3
7	3.3 - 8.3	260	259-261	19.1	248	244-249	17.6	36.7
8	10.3 - 15.3	240	237-242	19.3	214	210-215	17.1	36.4
9	17.3 - 22.3	206	203-208	19.1	181	179-182	17.1	36.2
10	24.3 - 29.3	235	233-236*	19.4	221	202-225	17.7	37.1
Average ± st.dev. Test cycles 6-10		241 ± 23	-	19.2 ± 0.1	222 ± 28	-	17.5 ± 0.5	36.7 ± 0.5
11	7.4 – 12.4	81	78-83	8.7	91	88-92	8.1	16.8

* The UV intensity sensor was cleaned prior to measuring.

Characterization of water from filter backflushing

On day 0 in test cycle 5 (brackish water) and test cycle 7 (seawater), the backflush-water from the filter was sampled and analysed. Results are shown in **Table 14**.

Table 14 Characterization of water from filter backflushing in test cyles 5 and 7

Parameter	Unit	Test cycle 5 (brackish water)	Test cycle 7 (seawater)
Temperature	ºC	4.6	3.3
pH	-	7.9	8.1
Dissolved oxygen	mg O_2/l	9.4	9.3
Salinity	PSU	21.2	32.2
Turbidity	FNU	55	80.5
Total suspended solids (TSS)	mg/l	103	91.0
Total organic carbon (TOC)	mg/l	21.6	32.0
Dissolved organic carbon (DOC)	mg/l	6.1	3.9
Particulate organic carbon (POC)	mg/l	24.9	31.8
Organic contents of TSS (ignition loss)	%	57.3	81.6
Total dry matter (TDM)	g/l	27.3	39.3
Density	g/ml	1.012	1.020

Because the total amounts of suspended material in the samples were low, determination of settleability was replaced by determination of total dry matter (TDM). The method used was according to EN 12880 (see paragraph 7.2.1 in NS EN 14702-1 described in 2.8.1 of this report). The content of salts provided the dominant contribution of the total amount of dry matter and of the density measurements of the sludge samples.

The results of these measurements indicated the efficiency of the filter for suspended solids removal. The concentration of TSS in the brackish water sludge sample was approximately two times higher than in the influent water (see table 7). The concentration of TSS in the seawater sludge sample was approximately six times higher than in the influent water samples (see table 8). Both for seawater and brackish water sludge samples, but mainly for seawater samples, organic particles were the dominant part of the total suspended solids (TSS) with 81.6% and 57.3% organic content of TSS in seawater and brackishwater sludge samples, respectively.

3.4 Effects of treatment and storage on the chemical water quality

3.4.1 Chemical water quality after treatment and during storage

The levels of total suspended solids (TSS), particulate organic carbon (POC), dissolved organic carbon (DOC) and UV-transmission during ballasting and after the five days storage period and deballasting are shown in **Appendix 1 (Table 1)** for all test cycles. The results after deballasting for both treated and control water are presented in **Table 15**. Changes in concentrations were observed during ballasting and during storage and deballasting. The largest consistently observed changes occurred for the particulate matter during storage and deballasting of brackish water (cycles 1-5); 59-63 % reduction in TSS and 65-72 % reduction in POC for the treated water compared to initial test water. However, the changes occurring during storage and deballasting of the control brackish water were similar (47-65 % reduction in TSS and 53-80 % reduction in POC) indicating that sedimentation during storage was the main mechanism for the observed reduction in particulate matter. Neither for the brackish water test cycles nor the seawater test cycles there were any significant reduction in particle concentration between treated water and control water during ballasting. This is due to the small size of the dominant particle load, passing the mesh openings of the filter unit. Particle load as turbidity in untreated, treated, and control water on day 0 and day 5 is shown in **Appendix 1 (Table 2).**

The relative change in DOC were probably insignificant during ballasting, but was reduced by 13-21% during the five days storage in the brackish water test cycles (1-5). However, the changes occurring during storage and deballasting of the control brackish water were similar; 5-19% reduction in DOC. The relative changes occurring during the storage of the less DOC-concentrated treated seawater (cycles 6-11) appeared to be somewhat smaller than what was observed for the brackish water. But again, no significant differences were observed between treated water and control water.

Table 15 Average concentration and standard deviation of triplicate samples of DOC, POC, TSS and UV-T in the treated and control water after 5 days storage and deballasting

	Treated water				**Control water**			
	DOC [mg C/l]	**POC [mg C/l]**	**TSS [mg/l]**	**UV-T %**	**DOC [mg C/l]**	**POC [mg C/l]**	**TSS [mg/l]**	**UV-T %**
Brackishwater test cycles								
1	4.4 ± 0.1	1.1± 0.1	13.7 ± 0.3	76.7 ± 0.3	4.9 ± 0.2	1.2 ± 0.1	15.4 ± 0.4	74.3 ± 0.3
2	4.2 ± 0.1	1.4 ± 0.1	15.7 ± 0.5	76.9 ± 0.3	4.6 ± 0.1	2.3 ± 0.2	22.8 ± 0.7	74.2 ± 0.7
3	4.4 ± 0.1	1.3 ± 0.0	17.2 ± 0.5	76.1 ± 0.9	4.7 ± 0.3	1.4 ± 0.0	19.5 ± 2.4	73.4 ± 0.6
4	4.4 ± 0.1	1.2 ± 0.1	17.1 ± 0.9	74.2 ± 1.0	4.4 ± 0.3	1.3 ± 0.0	17.5 ± 0.9	74.8 ± 0.3
5	4.3 ± 0.1	1.1 ± 0.1	19.2 ± 0.9	75.2 ± 0.4	4.1 ± 0.1	1.3 ± 0.2	21.2 ± 3.6	75.2 ± 0.4
Seawater test cycles								
6	2,0 ± 0,1	0,6 ± 0,1	7,3 ± 1,2	92,8 ± 0,1	1,9 ± 0,1	0,9 ± 0,1	6,5 ± 0,4	92,5 ± 0,3
7	1,9 ± 0,1	1,2 ± 0,1	8,2 ± 1,2	92,0 ± 0,3	2,0 ± 0,1	0,8 ± 0,0	7,3 ± 0,4	91,0 ± 0,6
8	1,8 ± 0,0	0,8 ± 0,0	8,9 ± 3,2	91,4 ± 0,2	1,8 ± 0,1	0,7 ± 0,1	8,2 ± 0,9	90,8 ± 0,6
9	2,1 ± 0,1	0,6 ± 0,1	7,8 ± 1,2	90,5 ± 0,4	2,0 ± 0,1	0,9 ± 0,0	12,6 ± 3,0	90,8 ± 0,5
10	2,4 ± 0,0	1,9 ± 0,0	16,1 ± 2,9	86,6 ± 0,2	2,3 ± 0,1	3,3 ± 0,4	17,2 ± 4,8	87,5 ± 0,5
11	2,3 ± 0,1	1,2 ± 0,0	10,1 ± 1,1	87,7 ± 0,2	2,5 ± 0,1	2,4 ± 0,4	11,2 ± 4,4	89,0 ± 0,3

The measured temperatures, pH, dissolved oxygen concentrations, salinities and total residual oxidants (measured as free and total chlorine) before treatment on day 0, during the five days storage and after deballasting on day 5 for all 11 test cycles and both treatment cycle and control cycle are shown in **Appendix 2**. In general, only minor changes were observed for these parameters during the course of each test cycle. The largest relative changes occurred in temperature during storage, but the change was in all cases less than one degree celcius during the five days storage period. The levels of total residual oxidants were below the detection limit (0.02 mg Cl/l) in all samples.

3.4.2 Disinfection by-products

A range of disinfection by products (DBPs) were analysed in the first two test cycles for each water quality in treated water, and in the first test cycle both in influent water and control water on day 0 and after five days storage (day 5). See **Table 16** and **Table 17**. None of the specific DBPs listed in Table 16 were detected at levels exceeding the detection limits in treated water. Only the group parameter adsorbable organic halogens (AOX) were observed at detectable levels in treated brackish water at day 5, but at similar levels as observed in control water after day 5.

Table 16 Measured concentration average and range of disinfection by-products in the two first brackish water test cycles (nr 1 and 2) on day 0 and after five days storage (day 5)

Disinfection by-products	**Unit**	**Detection**	**Influent**	**Treated**		**Control**	
Parameter/ Time (day)		**Limit**	**0**	**0**	**5**	**0**	**5**
Trichloromethane (chloroform)	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Bromodichloromethane	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Dibromochlorormetane	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Tribromomethane (bromoform)	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Chloroacetic acid (MCAA)	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
Dichloroacetic acid (DCAA)	µg/l	0.3	<0.30	<0.30	<0.30	<0.30	<0.30
Trichloroacetic acid (TCAA)	µg/l	0.2	<0.20	<0.20	<0.20	<0.20	<0.20
Bromoacetic acid (MBAA)	µg/l	0.2	<0.20	<0.20	<0.20	<0.20	<0.20
Dibromoacetic acid (DBAA)	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Bromochloroacetic acid (BCAA)	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Dichlorobromoacetic acid (DCBAA)	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Dibromochloroacetic acid (DBCAA)	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Tribromoacetic acid (TBAA)	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Dichloroacetonitrile	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Trichloroacetonitrile	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
2,4-Dibromophenol	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
2,6-Dibromophenol	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
2,4,6-Tribromophenol	µg/l	0.1	**0.39**	<0.10	<0.10	<0.10	<0.10
1,2-Dibromoethane	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
1,2,4-Tribromobenzene	µg/l	1.0	<1.0	<1.0	<1.0	<1.0	<1.0
1,2,3-Trichloropropane	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
2-Chlorotoluene	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
4-Chlorotoluene	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
1,2-Dibromo-3-chloropropane	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
1,2,3-Tribromobenzene	µg/l	1.0	<1.0	<1.0	<1.0	<1.0	<1.0
1,3,5-Tribromobenzene	µg/l	1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Monobromoacetonitrile	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Dibromoacetonitrile	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Bromochloroacetonitrile	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Dibromomethane	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
AOX	mg/l	0.020	<0.020	<0.020	**0.018** **<0.020-0.037**	<0.020	**0.010** **<0.020-0.021**
EOX	mg/l	0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Bromate	µg/l	1.0	<1.0	<1.0	<1.0	<1.0	<1.0

Table 17 Measured concentration average and range of disinfection by products in the two first seawater water test cycles (nr 6 and 7) on day 0 and after five days storage (day 5) in influent, treated and control waters

Disinfection by-products	**Unit**	**Detection limit**	**Influent**	**Treated**		**Control**	
Parameter/ Time (day)			**0**	**0**	**5**	**0**	**5**
Trichloromethane (chloroform)	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Bromodichloromethane	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Dibromochlorormetane	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Tribromomethane (bromoform)	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Chloroacetic acid (MCAA)	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
Dichloroacetic acid (DCAA)	µg/l	0.3	<0.30	<0.30	<0.30	<0.30	<0.30
Trichloroacetic acid (TCAA)	µg/l	0.2	<0.20	<0.20	<0.20	<0.20	<0.20
Bromoacetic acid (MBAA)	µg/l	0.2	<0.20	<0.20	<0.20	<0.20	<0.20
Dibromoacetic acid (DBAA)	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Bromochloroacetic acid (BCAA)	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Dichlorobromoacetic acid (DCBAA)	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Dibromochloroacetic acid (DBCAA)	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Tribromoacetic acid (TBAA)	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Dichloroacetonitrile	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Trichloroacetonitrile	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
2,4-Dibromophenol	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
2,6-Dibromophenol	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
2,4,6-Tribromophenol	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
1,2-Dibromoethane	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
1,2,4-Tribromobenzene	µg/l	1.0	<1.0	<1.0	<1.0	<1.0	<1.0
1,2,3-Trichloropropane	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
2-Chlorotoluene	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
4-Chlorotoluene	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
1,2-Dibromo-3-chloropropane	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
1,2,3-Tribromobenzene	µg/l	1.0	<1.0	<1.0	<1.0	<1.0	<1.0
1,3,5-Tribromobenzene	µg/l	1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Monobromoacetonitrile	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Dibromoacetonitrile	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Bromochloroacetonitrile	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Dibromomethane	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
AOX	mg/l	0.020	<0.020	<0.020	<0.020	<0.020	<0.020
EOX	mg/l	0.010	<0.010	<0.010	<0.010	**0.025**	<0.010
Bromate	µg/l	1.0	<1.0	<1.0	<1.0	<1.0	<1.0

3.5 Effects of treatment on organisms ≥50 µm in minimum diameter

The number of viable organisms ≥50 µm in minimum diameter, as determined on the basis of motility and integrity by microscope examination in treated test water and control immediately after treatment and after five days of storage, is shown in **Table 18.** Since it may take several hours for the lethal actions of the UV irradiation to show effect on motility of the exposed organisms, treated water samples with observable live organisms were always recounted after 24 hours both for samples on day 0 and day 5. Treated sample in the test 11 on day 0 show initially quite high viable counts. This was due to the presence of small naupli and rotatoria that was not retained by the filter. However, a recount of the samples after 24 hours showed that the UV treatment was effective.

For the performance of the ballast water treatment system to pass the regulation D-2 of the IMO guidelines, as shown in **Table 3,** less then 10 viable organisms ≥50 µm per m^3 should be present in the treated water after five days storage. This was met in all test cyles after treatement on day 5, even in samples analysed directly after treatment. The recount after 24 hours showed equal or less viable organisms than when observed immediately.

Table 18 Viable organisms ≥50 µm in minimum diameter in treated test water and control immediately after treatment and after five days of storage. Green background indicates that the required level was fulfilled, yellow background partial fulfilment, while red background indicates failure to fulfil required level. (a.d. = after deballasting on day 5). Values in brackets are recounts performed 24 hours after sampling

	Treated water		**Control water**	
	Day 0	**Day 5 (a.d)**	**Day 0**	**Day 5 (a.d.)**
Organisms ≥50 µm in minimum diameter (individuals m^{-3})				
Requirement	-	<10	-	>100
Test cycle 1	4.7 ± 1.2 (1.0 ± 1.0)	2.3 ± 2.1 (1.0 ± 1.7)	109620 ± 21034 (86698 ± 18603)	24083 ± 10678 (19250 ± 9128)
Test cycle 2	19.3 ± 4.9 (0)	1.0 ± 0.0 (0)	107024 ± 29121 (83483 ± 26541)	33085 ± 15331 (30141 ± 14861)
Test cycle 3	13.0 ± 1.7 (2.3 ± 1.2)	1.7 ± 1.2 (0.3 ±0.6)	102056 ± 12816 (84224 ± 16181)	17139 ± 4495 (14539 ± 4046)
Test cycle 4	14.0 ± 2.6 (0)	0.7 ± 0.6 (0.7 ± 0.6)	113933 ± 6407 (102100 ± 14264)	32613 ± 149 (28035 ± 3804)
Test cycle 5	27 ± 7.0 (1.7 ± 0.6)	4.0 ± 2.0 (0.3 ± 0.6)	71889 ± 13757 (65981 ± 13895)	34320 ± 10214 (294549 ± 9000)
Test cycle 6	2.3 ± 1.2 (0)	0.7 ± 1.2 (0)	56726 ± 12189 na	21758 ± 3961 (16115 ± 2846)
Test cycle 7	3.7 ± 2.1 (1.0 ± 1.0)	0.7 ± 0.6 (0.3 ± 0.6)	74522 ± 27781 (60933 ± 22679)	41868 ± 3386 (30532 ± 4335)
Test cycle 8	9.0 ±1.7 (2.3 ± 1.0)	8.0 ± 1.7 (3.0 ±1.0)	51469 ± 14758 (39046 ± 11175)	23846 ± 10919 (18404 ± 10738)
Test cycle 9	10.0 ± 2.6 (0)	2.3 ± 0.6 (0.7 ± 1.2)	51919 ± 9871 (34035 ± 6078)	20861 ± 1137 (14454 ± 735)
Test cycle 10	3.7 ± 2.1 (1.0 ± 1.0)	0.7 ± 0.6 (0.3 ± 0.6)	76587 ± 35435 (58487 ± 24329)	19365 ± 2130 (na)
Test cycle 11	448 ± 295 (0)	0.7 ± 1.2 (0)	77452 ± 15990 (62296 ± 11553)	23407 ± 5179 (16181 ± 2560)

na – not available

3.6 Biocidal effects on organisms ≥10-50 µm in minimum diameter

Less than 10 viable organisms per ml should be present in the treated water after five days storage for the BWMS to pass the regulation D-2 of the IMO guidelines (see **Table 3)**. The number of viable organisms ≥10-50 µm in minimum diameter, as determined by the serial dilution method in algal growth medium, by microscopy examination after incubation with CFDA-AM and by plate count, are shown in **Table 19, Table 20 and Table 21,** respectively, for treated test water and control water immediately after ballasting and after five days of storage and deballasting. Both the serial dilution method and the plate count method indicate that the Auramarine Crystal Ballast BWMS fulfilled the requirements in all test cycles, even the 11th test cycle with reduced UV irradiation. But the vital staining with CFDA-AM indicates that the required level of <10 viable organisms per ml were not reached for any of the brackish water cycles (1-5) nor for the seawater test cycle with reduced UV irradiation (cycle 11).

The two methods that indicate that the D-2 requirement was fulfilled are both based on cultivation, hence, a direct measure of the ability of the organisms to reproduce. The vital staining method is based on cell activity, which depends on a non-damaged cell membrane and the presence of necessary enzymes. Damage to the cell's DNA is a typical result of UV irradiation and it may take several hours, or even days, depending on e.g. the temperature, before this latent damage is expressed as a non-functioning cell. Therefore vital staining will give false positive numbers when the UV treatement is less than required to kill the cells immediately. Hence, the CFDA-AM staining was performed 24 hours after exposure and after the cells had been stored at 4 ^{o}C in the dark. However, the apparently weak effect of the treatment at day 0 (65-79 % reduction or 0.46-0.68 $\log_{10}$ reduction) and the apparent stronger effect of five days storage and the second treatment (95-99 % reduction or 1.34-1.81 $\log_{10}$ reduction) suggest that the 24 hour incubation before staining may have been too short. Therefore, the dilution culture method and plate count should be relied on when vital staining give uncertain results. It should also be noted that cells observed to be "alive" using vital staining on day 5, were with a few exceptions, always *Tetraselmis sp*. (see appendix I regarding details). Any actual viable *Tetraselmis sp*. would always be observed in the diltion cultures. The total viable number of *T. suecica* on day 5 determined by the dilution method, and the count of species other than *T. suecica* by the CFDA method, are summarized in Table 22.

As it is indicated from the results shown in Tables 19-22, the D-2 requirements of ≥100 cells/ml in the control water was met in all test cycles.

This taken into account, we conclude that the Auramarine Crystal Ballast BWMS fulfilled the D-2 requirement regarding biocidal effect on organisms ≥10-50 µm in minimum diameter.

Table 19 Viable organisms ≥10-50 µm in minimum diameter in treated test water and control immediately after treatment and after five days of storage determined by the serial dilution method. Green background indicates fulfilment. (a.d.= after deballasting on day 5)

	Treated water		Control water	
	Day 0	**Day 5 (a.d.)**	**Day 0**	**Day 5 (a.d.)**
Organisms ≥10-50 µm in minimum diameter (individuals ml^{-1})				
Requirement	-	<10	-	>100
Dilution method (average and 95 % confidence interval)				
Test cycle 1	50	0.2	5000	1600
	20-200	<0.1-1.1	2000-24000	600-5300
Test cycle 2	160	0.2	2000	2400
	60-530	<0.1-1.1	1000-14000	1000-9500
Test cycle 3	>240	0.4	2000	1600
	100-950	0.1-1.7	1000-14000	600-5300
Test cycle 4	>240	0.2	2000	1600
	100-950	<0.1-1.1	1000-14000	600-5300
Test cycle 5	>240	<0.2	1100	900
	100-950	<0.1-1.1	300-4800	300-3000
Test cycle 6	0.2	<0.2	2000	2400
	<0.1-1.1	<0.1-1.1	1000-14000	1000-9500
Test cycle 7	0.7	<0.2	5000	1600
	0.2-2.1	<0.1-1.1	2000-24000	600-5300
Test cycle 8	3.0	0.4	2000	2400
	1-12	0.1-1.5	1000-14000	1000-9500
Test cycle 9	0.4	0.2	5000	1600
	0.1-1.5	<0.1-1.1	2000-24000	600-5300
Test cycle 10	5.0	<0.2	2000	2400
	2-15	<0.1-1.1	1000-14000	1000-9500
Test cycle 11	>240	1.3	5000	2400
	100-950	0.5-3.9	2000-24000	1000-9500

Table 20 Viable organisms ≥10-50 µm in minimum diameter in treated test water and control immediately after treatment and after five days of storage determined by microscopy examination after incubation with CFDA-AM. (a.d.= after deballasting on day 5)

	Treated water		Control water	
	Day 0	**Day 5 (a.d.)**	**Day 0**	**Day 5 (a.d.)**
Organisms ≥10-50 µm in minimum diameter (individuals ml^{-1})				
Requirement	-	<10	-	>100
Microscope counts				
Test cycle 1	408 ± 114	40 ± 5	1971 ± 196	1711 ± 15
Test cycle 2	492 ± 87	19 ± 6	1737 ± 182	1050 ± 173
Test cycle 3	414 ± 24	58 ± 9	1806± 246	1361 ± 40
Tect cycle 4	869 ± 262	38 ± 3	869 ± 262	830 ± 230
Test cycle 5	885 ± 54	22 ± 2	2541 ± 144	1426 ± 65
Test cycle 6	119 ± 24	0	2265 ± 261	2036 ± 442
Test cycle 7	72 ± 30	0	1919 ± 319	1867 ± 79
Test cycle 8	85 ± 23	0	1625 ± 195	1517 ± 54
Test cycle 9	348 ± 58	0	1469 ± 249	2204 ± 26
Test cycle 10	284 ± 90	0	1789 ± 93	2113 ± 123
Test cycle 11	1469±232	348 ± 22	2014 ± 272	2982 ± 295

Table 21 Viable organisms ≥10-50 µm in minimum diameter in treated test water and control immediately after treatment and after five days of storage determined by plate count. Green background indicates fulfilment, yellow background indicates partial fulfilment and red background indicates lack of fulfilment. (a.d.= after deballasting on day 5)

	Treated water		**Control water**	
	Day 0	**Day 5 (a.d.)**	**Day 0**	**Day 5 (a.d.)**
Organisms ≥10-50 µm in minimum diameter (individuals ml^{-1})				
Requirement	-	<10	-	>100
Plate counts				
Test cycle 1	93 ± 15	<10	1690 ± 215	1590 ± 87
Test cycle 2	277 ± 6	<10	1983 ± 215	957 ± 155
Test cycle 3	307 ± 50	<10	1900 ± 144	1543 ± 240
Tect cycle 4	180 ± 40	<10	2053 ± 71	1073 ± 240
Test cycle 5	330 ± 17	<10	2100 ± 85	1683 ± 320
Test cycle 6	<10	<10	1617 ± 86	1980 ± 215
Test cycle 7	<10	<10	2080 ± 265	1850 ± 30
Test cycle 8	<10	<10	1377 ± 49	1333 ± 76
Test cycle 9	<10	<10	1327 ± 144	1490 ± 30
Test cycle 10	<10	<10	1677 ± 103	2053 ± 144
Test cycle 11	317 ± 49	<10	1853 ± 55	1850 ± 30

Table 22 Viable *T. suecica* from dilution cultures and counts of species other than *T. suecica* by the CFDA method after five day in treated test water. Total viable organisms ≥10-50 µm in minimum diameter was determined by adding viable CFDA-counts other than *T. suecica* to the number of viable *T. suecica* cells found in the serial dilution cultures

Test cycle	**Average viable *T. suecica* from dilution cultures (cell/ml)**	**Average counts of viable cells other than *T. suecica* by the CFDA method (cell/ml)**	**Total average count (cell/ml)**
Test cycle 1	0.2	0	0.2
Test cycle 2	0.2	0	0.2
Test cycle 3	0.4	0	0.4
Test cycle 4	0.2	0	0.2
Test cycle 5	<0.2	0.3 (*Thalassiosira sp.*)	0.3
Test cycle 6	<0.2	0	<0.2
Test cycle 7	<0.2	0	<0.2
Test cycle 8	0.4	0	0.4
Test cycle 9	0.2	0	0.2
Test cycle 10	<0.2	0	<0.2
Test cycle 11	1.3	1.6(*Thalassiosira sp.*) 0.3 (*Navicula sp.*)	3.2

3.7 Bactericidal effects

Table 23 shows the numbers of heterotrophic bacteria, coliform bacteria, *E.coli, Vibrio* spp., *Vibrio cholerae, Enterococcus* group and intestinal *Enterococci* in treated water and control water after ballasting at day 0 and after deballasting at day five. Regulation D-2 requires <250 cfu of *E.coli* per 100 ml, <1 cfu of *Vibrio cholera* (toxicogenic serotypes O1 and 0139) per 100 ml and <100 cfu of intestinal *Enterococi* per 100 ml in treated water at discharge at day 5. These requirements were fulfilled for all these bacterial species in all test cycles.

Vibrio spp. are common in salt and brackish surface water. However, the pathogenic serotypes O1 and O139 have never been isolated from Norwegian coastal water. *Vibrio cholera* was not found in any of the samples from treated water during the 11 test cycles.

The number of heterotrophic bacteria in the treated samples after ballasting on day 0 and on day 5 was reduced by 1-2 log units and 3-5 log respectively relative to the levels in control water. The counts in treated water on day 5 were below 100 cfu/ml in all cycles, except in cycle 9 with a count of 390 cfu/ml.

Table 23 Heterotrophic bactera, coliform bacteria, *E.coli*, *Vibrio* spp. *Vibrio cholera*, Enterococcus group and *intestinal Enterococci* in treated test water and control water after ballasting and after deballasting at day five for test cycles 1-11. Green background indicates fulfilment, yellow background indicates partial fulfilment and red background indicates lack of fulfilment. (a.d.= after deballasting on day 5)

	Treated water			**Control water**	
	Day 0	**Day 5 (a.d.)**		**Day 0**	**Day 5 (a.d.)**
Marine heterotrophic bacteria (cfu ml^{-1})					
Requirement	-	-		-	-
Test cycle 1	37 ± 11	61 ± 5		$4.7 \pm 1.9 \times 10^4$	$4.2 \pm 1.7 \times 10^5$
Test cycle 2	$2.7 \pm 0.5 \times 10^2$	54 ± 9		$1.2 \pm 0.04 \times 10^4$	$3.3 \pm 0.2 \times 10^5$
Test cycle 3	$8.7 \pm 1.8 \times 10^2$	15 ± 3		$9.2 \pm 1.8 \times 10^3$	$1.1 \pm 0.4 \times 10^6$
Test cycle 4	$2.4 \pm 0.3 \times 10^3$	29 ± 5		$1.3 \pm 0.3 \times 10^4$	$6.1 \pm 2.8 \times 10^5$
Test cycle 5	$7.3 \pm 1.7 \times 10^3$	43 ± 4		$2.7 \pm 0.3 \times 10^4$	$5.3 \pm 0.4 \times 10^5$
Test cycle 6	$1.2 \pm 0.6 \times 10^3$	80 ± 20		$1.3 \pm 1.1 \times 10^4$	$6.2 \pm 0.9 \times 10^5$
Test cycle 7	$1.9 \pm 0.2 \times 10^3$	29 ± 18		$1.7 \pm 0.3 \times 10^4$	$1.4 \pm 0.4 \times 10^5$
Test cycle 8	$1.4 \pm 0.1 \times 10^3$	63 ± 55		$1.8 \pm 0.2 \times 10^4$	$2.8 \pm 0.3 \times 10^4$
Test cycle 9	$3.1 \pm 0.6 \times 10^3$	$3.9 \pm 0.2 \times 10^2$		$1.4 \pm 0.3 \times 10^4$	$1.7 \pm 0.3 \times 10^5$
Test cycle 10	$4.2 \pm 0.5 \times 10^3$	32 ± 32		$2.1 \pm 0.4 \times 10^4$	$1.5 \pm 0.4 \times 10^5$
Test cycle 11	$1.0 \pm 0.3 \times 10^3$	80 ± 9		$1.7 \pm 0.2 \times 10^4$	$8.6 \pm 0.01 \times 10^4$
Coliform bacteria (Coli.) and *Escherichia coli (E. coli) (cfu 100 ml^{-1})**					
	Coli.	**Coli.**	***E. coli***	**Coli.**	**Coli.**
Requirement	-	-	<250*	-	-
Test cycle 1	<1	<1	<1	1.0 ± 0.0	0.7 ± 0.6
Test cycle 2	<1	<1	<1	<1	<1
Test cycle 3	<1	<1	<1	<1	<1
Test cycle 4	<1	<1	<1	<1	<1
Test cycle 5	<1	<1	<1	<1	<1
Test cycle 6	<1	<1	<1	1.0 ± 0.0	<1
Test cycle 7	<1	<1	<1	<1	<1
Test cycle 8	<1	<1	<1	<1	<1
Test cycle 9	<1	<1	<1	<1	<1
Test cycle 10	<1	<1	<1	<1	<1
Test cycle 11	<1	<1	<1	<1	<1
Vibrio* spp. and *Vibrio cholerae (*V. cholerae*) (cfu 100 ml^{-1})**					
	***Vibrio* spp.**	***Vibrio* spp.**	***V. cholerae***	***Vibrio* spp.**	***Vibrio* spp.**
Requirement	-	-	<1**	-	-
Test cycle 1	0.9 ± 0.9	35 ± 11	<1	$7.3 \pm 4.8 \times 10^3$	$1.9 \pm 0.3 \times 10^4$
Test cycle 2	48 ± 31	80 ± 5	<1	$2.9 \pm 1.0 \times 10^4$	$5.0 \pm 0.0 \times 10^4$
Test cycle 3	76 ± 59	42 ± 6	<1	$6.0 \pm 2.3 \times 10^2$	$1.2 \pm 0.01 \times 10^3$
Test cycle 4	16 ± 7	1.5 ± 1.4	<1	$4.9 \pm 0.9 \times 10^2$	$4.3 \pm 0.09 \times 10^2$
Test cycle 5	3.6 ± 0.9	4.8 ± 2.3	<1	$8.3 \pm 1.7 \times 10^3$	$1.6 \pm 0.3 \times 10^3$
Test cycle 6	13 ± 3	<1	<1	$1.2 \pm 0.3 \times 10^4$	$1.1 \pm 0.7 \times 10^3$
Test cycle 7	4.2 ± 1.9	<1	<1	$1.7 \pm 0.5 \times 10^3$	$4.8 \pm 2.3 \times 10^2$
Test cycle 8	3.9 ± 2.6	<1	<1	$1.4 \pm 0.1 \times 10^3$	$7.5 \pm 1.4 \times 10^2$
Test cycle 9	7.2 ± 1.9	<1	<1	$3.0 \pm 0.1 \times 10^2$	$5.0 \pm 0.9 \times 10^1$
Test cycle 10	1.8 ± 1.8	<1	<1	$1.0 \pm 0.1 \times 10^3$	$5.5 \pm 1.5 \times 10^2$
Test cycle 11	<1	<1	<1	$5.8 \pm 2.3 \times 10^1$	$3.3 \pm 2.1 \times 10^1$

Table 23 Continued

	Treated water			Control water	
	Day 0	Day 5 (a.d.)		Day 0	Day 5 (a.d.)
Enterococcus group (Ent. gr.) and Intestinal *Enterococci* (Int. *Ent.*)* (cfu 100 ml^{-1})**					
	Ent. gr.	**Ent. gr.**	**Int. *Ent.***	**Ent. gr.**	**Ent. gr.**
Requirement	-	-	<100***	-	-
Test cycle 1	<1	<1	<1	2.7 ± 0.6	1.7 ± 0.6
Test cycle 2	<1	<1	<1	4.0 ± 1.0	2.0 ± 1.7
Test cycle 3	<1	<1	<1	1.7 ± 0.6	11.7 ± 1.2
Test cycle 4	<1	<1	<1	1.0 ± 1.0	0.3 ± 0.6
Test cycle 5	<1	<1	<1	1.0 ± 1.0	<1
Test cycle 6	<1	2.0 ± 1.7	<1	3.0 ± 1.7	<1
Test cycle 7	<1	2.3 ± 0.8	<1	<1	<1
Test cycle 8	<1	<1	<1	<1	<1
Test cycle 9	<1	<1	<1	<1	<1
Test cycle 10	<1	<1	<1	<1	<1
Test cycle 11	<1	<1	<1	<1	<1

* The figures refer to the number identified as *Escherichia coli*/100 ml within the group of coliform bacteria. There is a requirement for *Escherichia coli* being <250 cfu/100ml after five days storage (D-2).
** The figures refer to the number identified as *Vibrio cholerae*/100 ml after five days storage. There is a requirement for toxicogenic *Vibrio cholerae* (serotypes O1 and O139) being <1 cfu/100 ml after five days storage (D-2).
*** The figures refer to the number identified as intestinal *Enterococci*/100 ml within the group of Enterococcus. There is a requirement for intestinal *Enterococci* being <100 cfu/100ml after five days storage (D-2).

3.8 Ecotoxicological responses

3.8.1 Fish toxicity testing

Both acute and chronic fish tests were performed with both treated brakish water and treated seawater. There were no observations of toxic effects in any of the tests (**Table 24**).

Table 24 Summary of the results for the fish test performed; acute and chronic tests with juvenile turbot (*Scopthalmus maximus*)

Fish Acute tests				
Test cycle	**Test id**	**Test sample**	**Result LC50 (%)**	**Comment**
Cycle 1	B702		> 100 %	
Cycle 6	B708		> 100 %	
Fish Chronic test				
			Result NOEC (%)	
Cycle 2-5	B702		≥ 100 %	
Cycle 6-9	B708		> 100 %	

3.8.2 Invertebrate toxicity testing

The invertebrate tests were performed using the copepod *Acartia tonsa*. This is a fairly sensitive species. No toxic effects were observed in any of the acute tests (**Table 2**5). In the reproduction tests with *Nitocera spinipes* mean reproduction was not significantly different between treated and control water.

Table 25 Test results of toxicity testing of ballast water treated with Auramarine BWMS tested with invertebrates; acute toxicity to *Acartia tonsa* and reproductive toxicity to *Nitocra spinipes*

Acute Invertebrate tests				
Test Cycle	**Test id**	**Test sample**	**Result NOEC %**	**Comment**
Cycle 1	B702		≥100 %	
Cycle 7	B710		≥100 %	
Chronic Invertebrate tests				
Cycles 3 and 4	B705		≥100 %	
Cycles 9 and 10	B713		≥100 %	

3.8.3 Growth inhibition of the marine alga *Skeletonema costatum*

A total of 6 algal growth inhibition tests were performed on treated water. The results are presented in **Table 26** with EC10 as the chronic endpoint and with EC50 as the acute endpoint.

Table 26 Effect concentrations (EC10 and EC50) as percentage treated ballast water diluted in control water

Test cycle	Test ID	Date	EC10 (%)	EC50 (%)
1	B702	25.01	>100	>100 %
4	B706	15.02	>100	>100 %
5	B707	22.02	>100	>100 %
6	B708	1.03	>100	>100 %
8	B711	16.3	>100	>100 %
9	B713	23.3	>100	>100 %

3.8.4 Oyster early life stage test

The oyster larvae test is a sensitive test, where it can be difficult to be sure if the observed effects are treatment related or is due to the general water quality of the test water. In the present test aged seawater was used as control and for diluting the treated water, while untreated ballast water (control water) was tested separately.

When testing brackish water in test cycle 1, all the oyster embryos died in the control. It is therefore not possible to evaluate the results from this test. In the test with seawater no effects were observed in neither treated nor non-treated ballast water (**Table 27**).

Table 27 Test results of toxicity testing of ballast water treated with Auramarine BWMS using oyster embryo

Chronic oyster embryo tests					
Test Cycle	**Test id**	**Test sample**	**Result LC50 %**	**Result NOEC (%)**	**Comment**
Cycle 1	B702			Can not conclude	Both control ballast water and treated water gave effects
Cycle 10	B714			100 %	

3.8.5 Reproduction test with rotatoria *Brachionus plicatilis*

The reproduction of *B. plicatilis* was tested both in brackish water and seawater at 5 concentrations in the range of 10 to 100 % dilutions of treated ballast water after discharge (**Table 28**). Statistical assessment of the observed reproduction indicated no significant effects.

Table 28 Test results of toxicity testing of ballast water treated with Auramarine BWMS using *Brachionus plicatilis*

Chronic rotifer tests					
Test Cycle	**Test id**	**Test sample**	**Result EC50 (%)**	**Result NOEC (%)**	**Comment**
Cycle 3	B705		>100 %	100 %	
Cycle 8	B711		>100 %	100 %	

3.8.6 Summary and conclusion with respect to toxicity of treated ballast water on discharge

A total of 18 toxicity tests with 6 different species and 5 different phyla have been performed. In the growth inhibition tests with algae with discharge water on day 5, no significant effects were observed in any of the tests. In acute and chronic tests with juvenile turbot (*Scopthalmus maximus*), no observations of toxic effects were observed. In the invertebrate tests performed using the copepod *Acartia tonsa*, no toxic effects were observed in any of the tests. The reproduction of *B. plicatilis* was tested both in brackish water and seawater at five different concentrations of treated ballast water after discharge. Statistical assessment of the reproduction indicated no significant effects. In the oyster larvae test, the observed toxic effects on the larvae in treated ballast water in the test cycle 1 could not be assumed to be caused by the BWMS as there was a 100 % lethal effect of untreated ballast water. No such effect was observed in neither treated nor untreated ballast water when testing with seawater.

The results of the toxicity testing indicate that treatment with Auramarine's Crystal Ballast BWMS should produce ballast water with no toxic effects upon discharge. It is therefore unlikely that the treated and discharged ballast water will have any adverse effect in the recipient water upon deballasting.

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Appendix 1.

Table 1 Chemical water quality (average and standard deviation of triplicate samples). Green background indicates that required level was fulfilled

TEST 1	**TSS mg/l**		**POC mg/l**		**DOC mg/l**		**UV-trans. %**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ = 16.5 kg**		**$POC_{Additive}$ = 7 kg**		**$DOC_{Additive}$ = 9.5 kg**			
Required level. WST	>50	-	>5	-	>5	-		
Influent water (WST)	50.7	0.9	5.6	0.4	5.5	0.3	66.0	0.1
Treated day 0 (TT2)	44.9	0.2	4.8	0.1	5.1	0.1	66.4	0.7
Treated day 5 (TT1)	13.7	0.3	1.1	0.1	4.4	0.1	76.7	0.3
Control day 0 (CT2)	48.4	3.5	5.7	0.7	5.2	0.1	66.7	0.2
Control day 5 (TT2)	15.4	0.4	1.2	0.1	4.9	0.2	74.3	0.3
TEST 2	**TSS mg/l**		**POC mg/l**		**DOC mg/l**		**UV-trans. %**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ = 18 kg**		**$POC_{Additive}$ = 7 kg**		**$DOC_{Additive}$ = 9.5 kg**			
Required level. WST	>50	-	>5	-	>5	-		
Influent water (WST)	53.5	1.6	5.8	0.1	5.0	0.1	64.4	0.8
Treated day 0 (TT2)	49.6	1.0	5.2	0.2	5.0	0.1	64.0	0.5
Treated day 5 (TT1)	15.7	0.5	1.4	0.1	4.2	0.1	76.9	0.3
Control day 0 (CT2)	47.9	0.7	5.4	0.3	5.0	0.1	64.1	0.6
Control day 5 (TT2)	22.8	0.7	2.3	0.2	4.6	0.1	74.2	0.7
TEST 3	**TSS mg/l**		**POC mg/l**		**DOC mg/l**		**UV-trans. %**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ =18 kg**		**$POC_{Additive}$ = 7 kg**		**$DOC_{Additive}$ = 9.5 kg**			
Required level. WST	>50	-	>5	-	>5	-		
Influent water (WST)	49.7	1.0	4.8	0.2	5.2	0.1	65.1	0.5
Treated day 0 (TT2)	46.8	0.6	4.7	0.0	5.5	0.2	63.7	0.5
Treated day 5 (TT1)	17.2	0.5	1.3	0.0	4.4	0.1	76.1	0.9
Control day 0 (CT2)	48.8	0.2	4.7	0.2	5.3	0.1	65.5	0.4
Control day 5 (TT2)	19.5	2.4	1.4	0.0	4.7	0.3	73.4	0.6
TEST 4	**TSS mg/l**		**POC mg/l**		**DOC mg/l**		**UV-trans. %**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ = 18 kg**		**$POC_{Additive}$ = 7 kg**		**$DOC_{Additive}$ = 9.5 kg**			
Required level. WST	>50	-	>5	-	>5	-		
Influent water (WST)	51.1	1.6	5.1	0.6	5.4	0.1	65.0	0.3
Treated day 0 (TT2)	47.2	1.0	4.5	0.1	5.4	0.1	64.3	0.2
Treated day 5 (TT1)	17.1	0.9	1.2	0.1	4.4	0.1	74.2	1.0
Control day 0 (CT2)	48.8	1.9	4.7	0.1	5.4	0.0	64.8	0.3
Control day 5 (TT2)	17.5	0.9	1.3	0.0	4.4	0.3	74.8	0.3
TEST 5	**TSS mg/l**		**POC mg/l**		**DOC mg/l**		**UV-trans. %**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ = 20 kg**		**$POC_{Additive}$ = 7 kg**		**$DOC_{Additive}$ = 9.5 kg**			
Required level. WST	>50	-	>5	-	>5	-		
Influent water (WST)	54.1	0.2	5.3	0.5	5.0	0.1	62.1	0.6
Treated day 0 (TT2)	51.9	0.9	4.9	0.2	5.0	0.1	61.4	0.2
Treated day 5 (TT1)	19.2	0.9	1.1	0.1	4.3	0.1	75.2	0.4
Control day 0 (CT2)	52.0	1.2	5.0	0.3	5.0	0.2	61.4	0.1
Control day 5 (TT2)	21.2	3.6	1.3	0.2	4.1	0.1	75.2	0.4
TEST 6	**TSS mg/l**		**POC mg/l**		**DOC mg/l**		**UV-trans. %**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ = 2.0 kg**		**$POC_{Additive}$ = 3.0 kg**		**$DOC_{Additive}$ = 2.5 kg**			
Required level. WST	>1	-	>1	-	>1	-		
Influent water (WST)	14.2	2.1	2.8	0.1	1.9	0.1	93.2	0.7

Treated day 0 (TT2)	12.6	1.0	2.3	0.0	2.0	0.0	93.0	0.2
Treated day 5 (TT1)	7.3	1.2	0.6	0.1	2.0	0.1	92.8	0.1
Control day 0 (CT2)	14.9	1.7	2.7	0.2	1.9	0.0	93.0	0.2
Control day 5 (TT2)	6.5	0.4	0.9	0.1	1.9	0.1	92.5	0.3
TEST 7	**TSS mg/l**		**POC mg/l**		**DOC mg/l**		**UV-trans. %**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ = 2.0 kg**		**$POC_{Additive}$ = 3.0 kg**		**$DOC_{Additive}$ = 2.5 kg**			
Required level. WST	>1	-	>1	-	>1	-		
Influent water (WST)	14.8	4.3	2.8	0.1	2.0	0.1	92.4	0.2
Treated day 0 (TT2)	12.6	0.8	2.3	0.1	2.0	0.0	92.1	0.4
Treated day 5 (TT1)	8.2	1.2	1.2	0.1	1.9	0.1	92.0	0.3
Control day 0 (CT2)	14.8	1.3	2.7	0.0	2.0	0.0	92.5	0.6
Control day 5 (TT2)	7.3	0.4	0.8	0.0	2.0	0.1	91.0	0.6
TEST 8	**TSS mg/l**		**POC mg/l**		**DOC mg/l**		**UV-trans. %**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ = 2.0 kg**		**$POC_{Additive}$ = 3.0 kg**		**$DOC_{Additive}$ = 2.5 kg**			
Required level. WST	>1	-	>1	-	>1	-		
Influent water (WST)	13.0	0.3	2.7	0.1	2.1	0.1	89.8	0.7
Treated day 0 (TT2)	13.0	0.8	2.7	0.5	2.0	0.1	88.2	0.8
Treated day 5 (TT1)	8.9	3.2	0.8	0.0	1.8	0.0	91.4	0.2
Control day 0 (CT2)	13.6	1.3	2.6	0.1	2.1	0.1	89.8	0.4
Control day 5 (TT2)	8.2	0.9	0.7	0.1	1.8	0.1	90.8	0.6
TEST 9	**TSS mg/l**		**POC mg/l**		**DOC mg/l**		**UV-trans. %**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ = 2.0 kg**		**$POC_{Additive}$ = 3.0 kg**		**$DOC_{Additive}$ = 2.5 kg**			
Required level. WST	>1	-	>1	-	>1	-		
Influent water (WST)	18.3	3.3	2.8	0.2	2.1	0.1	90.9	0.1
Treated day 0 (TT2)	14.7	0.9	2.5	0.1	2.1	0.1	90.0	0.2
Treated day 5 (TT1)	7.8	1.2	0.6	0.1	2.1	0.1	90.5	0.4
Control day 0 (CT2)	15.0	1.5	2.9	0.1	2.1	0.0	90.7	0.0
Control day 5 (TT2)	12.6	3.0	0.9	0.0	2.0	0.1	90.8	0.5
TEST 10	**TSS mg/l**		**POC mg/l**		**DOC mg/l**		**UV-trans. %**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ = 2.0 kg**		**$POC_{Additive}$ = 3.0 kg**		**$DOC_{Additive}$ = 2.5 kg**			
Required level. WST	>1	-	>1	-	>1	-		
Influent water (WST)	19.8	3.7	3.7	0.1	2.2	0.1	88.1	0.6
Treated day 0 (TT2)	17.2	2.5	3.1	0.1	2.6	0.1	86.9	0.3
Treated day 5 (TT1)	16.1	2.9	1.9	0.0	2.4	0.0	86.6	0.2
Control day 0 (CT2)	21.6	2.0	3.8	0.3	2.2	0.1	88.3	0.4
Control day 5 (TT2)	17.2	4.8	3.3	0.4	2.3	0.1	87.5	0.5
TEST 11	**TSS mg/l**		**POC mg/l**		**DOC mg/l**		**UV-trans. %**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ = 2.0 kg**		**$POC_{Additive}$ = 3.0 kg**		**$DOC_{Additive}$ = 2.5 kg**			
Required level. WST								
Influent water (WST)	17.0	4.6	3.0	0.1	2.8	0.1	88.7	0.2
Treated day 0 (TT2)	15.2	1.5	2.6	0.1	2.6	0.1	87.7	0.3
Treated day 5 (TT1)	10.1	1.1	1.2	0.0	2.3	0.1	87.7	0.2
Control day 0 (CT2)	17.8	4.8	2.9	0.1	2.7	0.1	88.4	0.2
Control day 5 (TT2)	11.2	4.4	2.4	0.4	2.5	0.1	89.0	0.3

Table 2 Average turbidity (NTU) with standard deviation (stdev) in untreated, treated, and control water on day 0 and day 5

Test nr	Tank	Day 0			Day 5		
		Average	Stdev	% stdev	Average	Stdev	% stdev
Test 1	**Influent**	20.2	0.2	0.9	-	-	-
	Treated	20.0	0.1	0.3	8.5	0.2	2.7
	Control	20.2	0.1	0.6	7.9	0.2	2.2
Test 2	**Influent**	21.9	0.6	3.0	-	-	-
	Treated	21.5	0.2	0.9	11.3	0.3	2.5
	Control	22.0	0.3	1.3	11.4	0.3	2.4
Test 3	**Influent**	22.5	0.1	0.6	-	-	-
	Treated	22.0	0.5	2.1	10.0	0.2	1.7
	Control	22.7	0.4	1.9	10.0	0.3	3.3
Test 4	**Influent**	20.7	0.1	0.3	-	-	-
	Treated	20.7	0.1	0.7	9.1	0.1	1.5
	Control	20.8	0.2	1.0	9.6	0.1	1.1
Test 5	**Influent**	23.8	0.3	1.1	-	-	-
	Treated	24.7	0.2	0.7	10.5	0.2	1.6
	Control	25.2	0.1	0.4	10.6	0.2	2.0
Test 6	**Influent**	4.9	0.1	1.1	-	-	-
	Treated	4.5	0.0	0.9	3.0	0.0	1.4
	Control	4.8	0.1	2.1	3.3	0.1	1.6
Test 7	**Influent**	4.3	0.1	1.9	-	-	-
	Treated	4.4	0.0	0.9	3.0	0.1	3.5
	Control	4.6	0.0	0.9	3.4	0.1	3.0
Test 8	**Influent**	4.8	0.1	2.6	-	-	-
	Treated	4.3	0.1	2.3	3.3	0.1	0.0
	Control	4.6	0.2	3.5	3.2	0.1	2.3
Test 9	**Influent**	4.7	0.1	2.3	-	-	-
	Treated	4.5	0.1	1.2	2.7	0.0	1.5
	Control	4.8	0.2	3.2	2.7	0.1	1.9
Test 10	**Influent**	4.5	0.1	2.4	-	-	-
	Treated	4.3	0.1	1.2	3.8	0.1	1.5
	Control	4.3	0.2	3.7	4.2	0.1	2.3
Test 11	**Influent**	4.1	0.1	2.0	-	-	-
	Treated	4.0	0.0	1.0	2.9	0.1	3.8
	Control	4.0	0.1	1.3	3.6	0.1	3.4

Appendix 2.

Table 1 Temperature, pH, dissolved oxygen, salinity and total residual oxidants (TRO) measured as free and total chlorine in the test water tank (WST) prior to treatment on day 0, during storage and after deballasting on day 5 for both treated and control water for each test cycle. (b.d = before deballasting. a.d = after deballasting)

Parameter		**WST**	**Treated water**					**Control water**				
	Day	**0**	**0**	**1**	**2**	**5 (b.d)**	**5 (a.d)**	**0**	**1**	**2**	**5 (b.d.)**	**5 (a.d)**
	Unit											
Test Cycle 1												
Temperature	ºC	4.60	4.50	4.40	4.60	4.7	4.50	4.40	4.30	4.10	4.1	4.00
pH	-	7.87	7.94	7.85	7.87	7.7	7.84	7.92	7.88	7.84	7.65	7.79
Dissolved O_2	mg/l	9.8	9.5	10.1	10.3	9.7	10.0	10.0	10.3	10.2	9.4	9.6
Salinity	PSU	21.2	21.2	21.2	21.2	21.1	21.2	21.2	21.3	21.2	21.2	21.2
Free chlorine	mg/l	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤0.02
Total chlorine	mg/l	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤0.02
Test Cycle 2												
Temperature	ºC	4.80	5.00	4.90	5.20	4	4.10	4.80	4.70	4.80	4.5	4.50
pH	-	7.81	7.91	7.94	7.91	7.81	7.78	7.94	7.96	7.93	7.74	7.72
Dissolved O_2	mg/l	9.8	9.6	9.0	8.8	9.1	9.4	9.8	9.0	9.7	8.9	10.0
Salinity	PSU	21.4	21.4	21.4	21.4	21.4	21.3	21.4	21.4	21.4	21.3	21.3
Free chlorine	mg/l	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤0.02
Total chlorine	mg/l	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤0.02
Test Cycle 3												
Temperature	ºC	3.80	4.00	4.10	4.10	3.9	3.90	3.80	3.90	4.00	4.2	4.20
pH	-	7.78	7.89	7.89	7.93	7.73	7.82	7.93	7.97	7.94	7.72	7.83
Dissolved O_2	mg/l	9.7	9.8	9.8	9.8	9.2	9.1	9.7	9.9	9.9	9	9.1
Salinity	PSU	21.3	21.2	21.2	21.2	21.2	21.1	21.2	21.2	21.2	21.1	21.1
Free chlorine	mg/l	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤0.02
Total chlorine	mg/l	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤0.02
Test Cycle 4												
Temperature	ºC	5.10	5.10	4.90	4.80	4.4	4.60	4.90	4.60	4.40	3.8	3.80
pH	-	7.71	7.82	7.82	7.78	7.61	7.64	7.83	7.84	7.81	7.66	7.60
Dissolved O_2	mg/l	8.6	8.6	8.6	9.0	8	8.2	8.8	8.9	9.0	8.3	8.6
Salinity	PSU	21.7	21.7	21.7	21.7	21.7	21.7	21.7	21.7	21.7	21.7	21.7
Free chlorine	mg/l	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤0.02
Total chlorine	mg/l	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤0.02
Test Cycle 5												
Temperature	ºC	4.60	4.80	4.80	4.90	4.5	4.40	4.60	4.60	4.70	4.4	4.30
pH	-	7.79	7.85	7.91	7.87	7.66	7.69	7.90	7.92	7.88	7.71	7.70
Dissolved O_2	mg/l	10.4	9.9	10.2	10.3	9.4	9.1	10.5	10.2	10.2	9.5	9.3
Salinity	PSU	21.2	21.2	21.1	21.1	21.1	21.1	21.2	21.1	21.1	21.1	21.1
Free chlorine	mg/l	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤0.02
Total chlorine	mg/l	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤0.02

Table 1 To be continued

Parameter	Day	WST	Treated water					Control water				
		0	0	1	2	5 (b.d)	5 (a.d)	0	1	2	5 (b.d.)	5 (a.d)
	Unit											
Test Cycle 6												
Temperature	ºC	5.90	5.90	5.90	6.00	6.3	6.20	5.80	5.70	5.70	5.9	6.00
pH	-	7.74	8.02	7.89	7.99	8.02	8.03	7.86	7.92	7.93	8.03	8.14
Dissolved O_2	mg/l	9.3	9.2	9.4	9.2	9.2	8.9	9.4	9.3	9.3	9.1	9.3
Salinity	PSU	32.1	32.1	32.1	32.0	32	32.0	32.1	32.1	32.0	32	32.0
Free chlorine	mg/l	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤0.02
Total chlorine	mg/l	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤0.02
Test Cycle 7												
Temperature	ºC	4.30	4.50	4.40	4.40	4.3	4.40	4.30	4.20	4.10	4.1	4.20
pH	-	8.06	7.92	7.89	7.90	7.89	8.02	7.93	7.91	7.90	7.92	8.01
Dissolved O_2	mg/l	9.1	8.9	9.1	9.3	9.2	9.0	8.9	9.2	9.3	9.2	9.3
Salinity	PSU	32.2	32.2	32.2	32.2	32.2	32.2	32.2	32.2	32.2	32.2	32.2
Free chlorine	mg/l	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤0.02
Total chlorine	mg/l	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤0.02
Test Cycle 8												
Temperature	ºC	5.3	5.5	5.7	5.6	5.3	5.4	5.4	5.5	5.5	5.2	5.2
pH	-	7.94	7.92	7.93	8.05	8.01	8.00	7.93	7.95	8.06	8.03	8.01
Dissolved O_2	mg/l	9.1	9.3	9.4	9.3	9.0	8.7	9.3	9.0	9.2	9.0	8.8
Salinity	PSU	32.3	32.2	32.2	32.2	32.2	32.2	32.2	32.2	32.2	32.2	32.2
Free chlorine	mg/l	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤0.02
Total chlorine	mg/l	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤0.02
Test Cycle 9												
Temperature	ºC	4.6	4.8	4.8	4.8	4.5	4.6	4.8	4.7	4.7	4.3	4.4
pH	-	8.11	8.09	8.10	8.08	7.97	7.98	8.1	8.08	8.09	8.00	8.02
Dissolved O_2	mg/l	9.5	9.2	9.6	9.6	9.4	9.2	9.4	9.6	9.5	9.3	9.5
Salinity	PSU	32.6	32.6	32.6	32.5	32.5	32.5	32.6	32.6	32.6	32.6	32.6
Free chlorine	mg/l	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤0.02
Total chlorine	mg/l	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤0.02
Test Cycle 10												
Temperature	ºC	5.50	5.70	5.70	5.60	5.3	5.40	5.60	5.60	5.50	5.2	5.20
pH	-	8.36	8.32	8.22	8.24	8.1	8.11	8.37	8.23	8.23	8.13	8.02
Dissolved O_2	mg/l	12.2	11.7	11.4	11.0	10.1	9.7	12.0	11.2	10.4	10.3	10.0
Salinity	PSU	32.1	32.1	32.0	32.0	32	32.0	32.1	32.0	32.1	32	32.0
Free chlorine	mg/l	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤0.02
Total chlorine	mg/l	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤0.02
Test Cycle 11												
Temperature	ºC	6.20	6.30	6.20	6.20	6.2	6.40	6.40	6.30	6.20	6.1	6.40
pH	-	8.06	8.13	8.07	8.07	8.01	8.02	8.19	8.08	8.09	8.02	8.03
Dissolved O_2	mg/l	9.9	9.7	9.9	9.6	9.4	9.5	9.9	10.0	9.7	9.2	9.3
Salinity	PSU	32.3	32.2	32.3	32.3	32.3	32.3	32.3	32.3	32.3	32.3	32.3
Free chlorine	mg/l	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤0.02
Total chlorine	mg/l	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤ 0.02	≤0.02